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SCIENCE

JULY 13, 1951

VOLUME 114 NUMBER 2950

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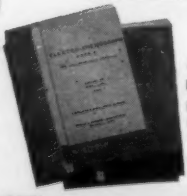

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Laboratory Infections

IN THE search for agents of infectious disease there have been occasional instances of self-inoculation to prove an etiological relationship between a given microorganism and the disease with which it was believed to be associated. More frequently, however, laboratory workers have become infected unintentionally by the agents with which they were working as a result of some accident or without the exact circumstances being recognized. Certain infectious agents, such as those of undulant fever, tularemia, psittacosis, and Q fever, were prone to infect the persons working with them.

With new infectious agents being recognized, with more workers being exposed to the danger of laboratory infection, and with new techniques and procedures being constantly introduced, there has been an increasing interest in the protection of laboratory personnel. To obtain more information regarding the occurrence of laboratory infection, the National Institutes of Health sponsored a survey, conducted by means of a questionnaire mailed to nearly 5,000 U. S. laboratories. It revealed 1,342 instances of presumed laboratory-acquired infection, with 39 deaths (*Am. J. Pub. Health* [in press]), only one third of which had been recorded previously in the literature. More than 70 different agents were involved, representing bacteria, viruses, fungi, rickettsiae, and protozoa. The diseases most frequently encountered were brucellosis, tuberculosis, and hepatitis, which together accounted for about one third of all infections. Workers come in contact with the agents of these diseases through the handling of cultures, the examination of specimens, and the processing of blood and blood derivatives. The latter constitutes the principal source of hepatitis. There are other agents, however, with which relatively few persons have laboratory contact. Yet the number of infections caused by them has been large. For example, tularemia, psittacosis, typhus and Q fever, and the fungus infection coccidioidomycosis occur as institutional outbreaks or as scattered cases in laboratories where these diseases are studied intensively.

The greatest hope of preventing infection lies in the recognition of the sources of infection. Only 16 per cent of all the recorded infections could be traced to a definite incident that resulted in contamination. These are, of course, preventable in the sense that all accidents are preventable. It is the unrecognized sources of infection that constitute the greatest problem. In many instances circumstantial evidence points to aerogenic transmission. Coccidioidomycosis, for example, is naturally acquired by inhalation of the fungus spores present in dust. When cultures of the fungus are opened, the dry spores are easily detached and may be inhaled not only by the worker but also by other persons in the same room or building. It is more difficult to understand the formation of aerosols from suspensions of viruses and rickettsiae, but recent studies demonstrate that aerosols are commonly released when infectious materials are transferred from one container to another. To diminish the chances of aerogenic transmission certain safety measures, such as the use of protective hoods and pipetting devices, have been recommended. Further protection against some infections is available in the form of specific immunization.

Laboratory-acquired infections do not always follow the pathways of transmission established for the naturally occurring disease. Yellow fever has developed in the absence of the vector; the virus of lymphogranuloma venereum has infected persons working with it in the laboratory in the absence of venereal contact. These examples should be of interest to those concerned with protection against biological warfare because they suggest that even in the absence of some of the links in the usual chain of transmission a given agent might be a potential danger if properly dispersed in the environment. The occupational hazards are now being recognized not only among workers in infectious disease laboratories but in other fields related to medicine.

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SCIENCE, founded in 1880, is published each Friday by the American Association for the Advancement of Science at the Business Press, 10 McGovern Ave., Lancaster, Pa. Entered as second-class matter at the Post Office at Lancaster, Pa., January 13, 1948, under the Act of March 3, 1879. Acceptance for mailing at the special rate postage provided for in the Act of February 28, 1925, embodied in Paragraph (d-2) Section 34.40 P. L. & R. of 1948.

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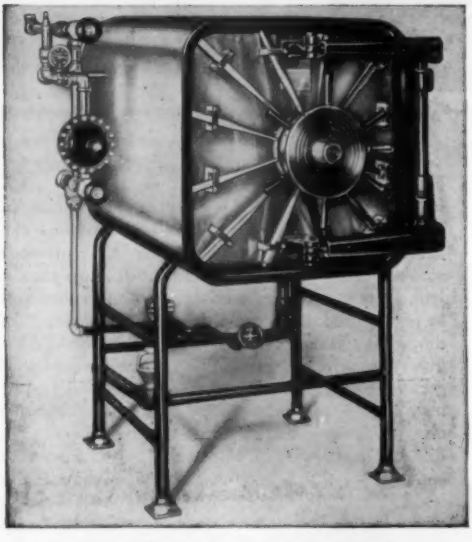

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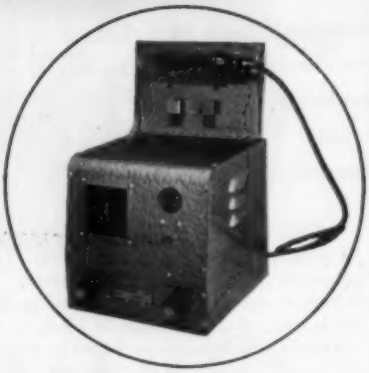

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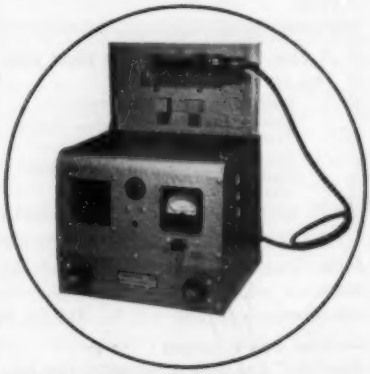

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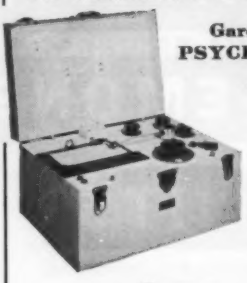

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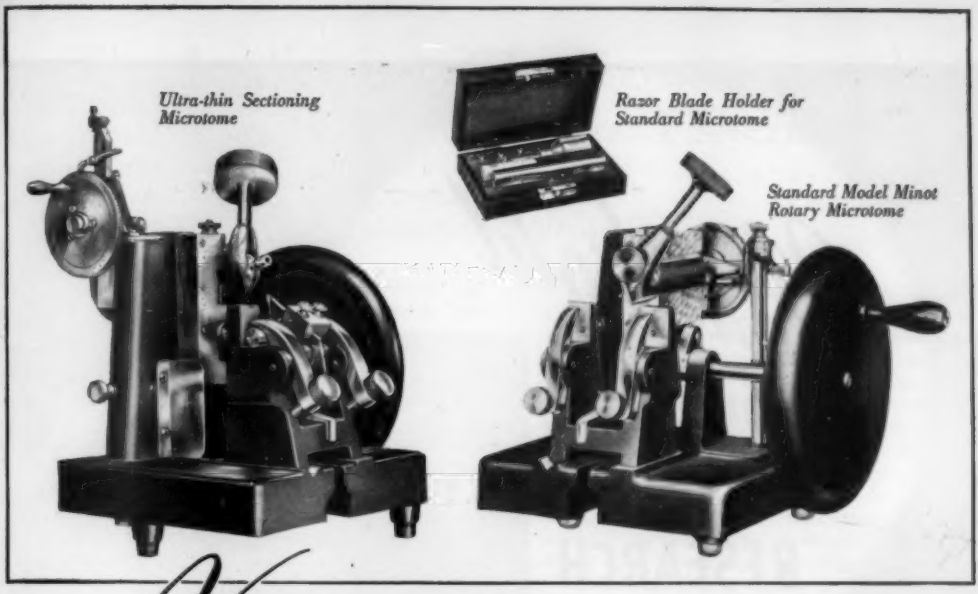

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The Application of Ecological Research to Aquiculture and the Fisheries[1]

Curtis L. Newcombe

U. S. Public Health Service, San Francisco, California

THERE IS A WIDESPREAD BELIEF that the era of a successful aquiculture is just around the corner; that farming the nation's ponds and lakes and the estuarine waters along the continental shores is quite as practical as the age-old practice of farming the soil. Without disparaging the invaluable aquicultural results that have accrued during the past decade or so, I think it must be stated frankly that major obstacles are still ahead of us. Lest we be discouraged, it may be well to remember that soil farmers are not altogether happy in their present situation, despite the highly specialized scientific agriculture that has evolved since the turn of the century. In 1939, of the six million or so farm families in the nation, 65 per cent grossed less than $1,000 cash annually. More than one million had a gross annual cash income of $250 or less (last available census figures). Competent agricultural opinion is that the relative picture today is probably similar to that of 1939.

I refer to the soil farmer, not only because aquatic biologists can learn much from his experience, but also because there is often a decided interdependence between him and the fisherman. Shortsighted farm practices have not only depleted soils of their natural productiveness, but they have silted up numerous rivers, lakes, streams, and bays throughout the nation, causing deterioration of the aquatic environment for economically important species. Watershed protection is probably the greatest single all-embracing need of the farmer and the fisherman alike. Natural spawning grounds for anadromous species in important West Coast streams have been destroyed or greatly reduced. Once-permanent streams are now seasonal. The imprint of pollution is accentuated with changing flows. On the Pacific Coast, perhaps the most striking example of wanton destruction by man-made erosion and pollution is San Francisco Bay. Here an oyster fishery has been extinguished, and the fin fisheries seriously reduced. On the Atlantic Coast, the productivity of the Chesapeake oyster grounds has likewise undergone irreparable damage. But the case of the soil farmer, historically, is quite different from that of the water farmer. The soil farmer *owns* his land, and his success is largely dependent on his diligence and ingenuity. Water, on the other hand, is usually publicly owned; most progress is being made where it is not. Witness the great strides made in the field of private pond culture in Alabama, and in oyster culture in Connecticut.

In large bodies of water, whether they be lakes, rivers, or oceans, there is the very real problem of multiple ownership *without* multiple responsibility. Interest rests primarily in today's catch with little thought of what remains for tomorrow. And this situation brings us to the crux of our discussion—namely, the application of ecological knowledge to human affairs. I am reminded of the introductory sentence in a paper entitled "Fish Management, Looking Forward," presented in 1937 by Carl Hubbs before the American Fisheries Society. It stated, "The need for thorough, soundly planned, and adequately administered fish management is coming to be generally appreciated." Referring to the past record of achievement, he went on to say, "It is being realized that the well-meaning efforts of the past have been to a large degree haphazard, incomplete, imperfect, uncoordinated, nontechnical, and untested." I believe the desideratum is here clearly indicated. Since 1937 important progress has been made in the fish management field. Technological staffs have been implemented, experimental research has become intensified, population analysis has been perfected, and new educational programs have been inaugurated.

The picture for fresh-water fish management is perhaps more promising than for salt-water management. I suppose the shortage of protein during the second world conflict played a major part in the impetus given oceanic fishery investigations since 1945. In any event, studies on a scale heretofore undreamed-of are in progress on the Pacific Coast of North America. One of these centers on the pilchard or sardine fishery, and I have elected to review events in the development of this fishery, first, because a crisis in it has offered a major challenge to administrators, biologists, and fishermen alike; second, because it is intrinsically an ecological problem; and third, because it is now presenting, although a little late, a classical example of a unified, balanced, cooperative effort in scientific management of a great marine resource. Let us hope that, when the full story is written, it will prove to be an analog of the halibut fishery of the North Pacific Coast, developed so skillfully under the leadership of W. F. Thompson.

A great deal has been written about the sardine since it became a major fishery during the first world war. Mention should be made of papers by Elmer Higgins, W. L. and E. C. Scofield, Frances N. Clark, R. B. Tibby, R. P. Silliman, O. E. Sette, L. A. Wal-

[1] Presented in the symposium of the Ecological Society of America on "The Application of Ecological Knowledge to Human Affairs," Columbus, Ohio, Sept. 12, 1950.

ford, E. H. Ahlstrom, and their associates, among the American workers that have done so much to assure possibilities for a permanent and profitable future for this industry.

I shall review briefly certain of the high lights in the utilization of this natural resource and the factors largely responsible for its disastrous decline from a peak California production of 726,000 tons in 1936–37 to a low of about 110,000 tons in the 1946–47 season. Also, I shall refer to certain ecological data that explain some fluctuations in the population.

During the 1918–19 season, the sardine canning industry yielded 75,000 tons. By 1936–37, it had become an oil business, and the tonnage was up to 726,000 tons. Profit in oil was responsible for the increase, and despite California policy that the state fish crop should be used as food for man, reduction plants multiplied, legislative efforts and those of a minority of the producers notwithstanding. Scofield points out that in the early thirties leading producers were supporting the constructive efforts of the State Division of Fish and Game, and it appeared that disaster would be averted and that a rational management of the sardine harvest would result. But this was not to be. Reduction ships started to operate on the high seas outside state jurisdiction, and the economic balance was again upset. Understandably, the state legislature would not curtail the established shore industry employing nearly 10,000 cannery workers while unregulated competition from reduction ships threatened to run the canners out of business.

Biologists at the Terminal Island Laboratory were quite well aware that a major catastrophe was imminent because the *catch per unit of effort* was on the decline, even though total catches continued to increase. Canneries that once handled 80 tons per day can now take 80 tons or more per hour. Numerous warnings were issued by the Division of Fish and Game as early as 1930 but, seemingly, research evidence was in itself insufficient proof.

The decline started in 1937–38 and continued with alarming consistency until the 1946–47 season, when the California cannery catch reached a low of approximately 110,000 tons. The monthly catch of an average vessel in 1946–47 was 50 per cent below that of the previous season, and in 1947–48 the catch again dropped 50 per cent below the 1946–47 take. Practically all the fish were used for canning. Prices of raw fish were high, whereas oil and meal prices were down. Studies up to 1948 pointed to two main reasons for the decline—a drop in abundance caused by extremely heavy fishing, and a simultaneous succession of many seasons of very poor spawning. This information had been available for several years and was repeatedly used as a scientific basis on which to warn the industry of its impending doom. Most of the industry had ignored the warning signals.

In the crisis, industry, state and federal administrators, and biologists took a decisive step. Industry appointed a Sardine Industry Advisory Committee to work with the State Bureau of Marine Fisheries. Two objectives were agreed upon: first, a temporary program of legislation embodying a size limit and a limitation in the number of fishing days and, second, a long-range legislative program of conservation.

Even at this crucial stage industry failed to go all out in the gamble for survival. It did support some very important and highly constructive recommendations of the State Bureau of Marine Fisheries. The summer canning industry, which draws upon immature fish, was eliminated by industry-supported legislation. Also, the seasons were shortened by one month. Extremely important was the aid given by industry to legislative financial support of a truly great scientific research program, now operating with an annual budget totaling more than half a million dollars.

What about the present status of the population? The cannery and reduction plant catch in 1946–47 was 110,000 tons, in 1947–48 it was 160,000 tons, and in 1948–49 the total was 335,000. The last catch would have been greater but for poor market conditions. Richard Croker, chief of the California Bureau of Marine Fisheries, has stated that, following 1939, there was not a really good spawning season until 1947, although there was some spawning in 1946. The 1947 year class grew exceptionally fast. Together with the 1946 year class, it was, according to Croker, responsible for about 80 per cent of the 335,000-ton catch in 1948–49. Some real benefit has also resulted from the restrictions on the season, as well as on the operation of the reduction plants at San Pedro and Monterey, and in the San Francisco Bay area.

As I have pointed out, this major economic crisis in the largest fishery in the Western Hemisphere resulted in what is destined to be one of the greatest battles, if not the greatest battle, on record for the cause of scientific fishery management and conservation. O. E. Sette, as early as 1943, proposed an admirable cooperative research program designed to determine how fishing affects the sardine resource. I think it is worthy of emphasis that no fewer than eight groups, including industry, one federal and three state agencies, two educational institutions, and the Dominion of Canada, are pulling together toward a common purpose. We have in this instance, then, a striking example of the application of biological knowledge to an economic problem. Thousands of plant workers and boat crews, from Lower California to British Columbia, are dependent on the industry, and the nation's supply of proteins and fats is influenced by the fishery.

Knowledge of how the ecological factors of the marine environment influence the size and composition of the catch is quite as important as an understanding of how fishing intensity affects the population. At present, there are two primary biological considerations in the sardine problem. One of these is the size of the year classes, which are now known to vary enormously. According to Croker,[2] the time between

[2] I am indebted to Richard Croker, and to J. L. McHugh, of the Scripps Institution of Oceanography, for reading this manuscript and providing valuable comments.

good or poor classes varies from one year to several. That brings us to the all-important question concerning the factors that cause the success or failure of a spawning. To answer this query the extensive oceanographic and biologic investigations now in progress were initiated.

In the interest of brevity, I shall refer only to the temperature and food factors in their relation to sardine production. One can scarcely avoid mention of the temperature factor in view of the enlightening conclusions reached by Hubbs in his analysis of temperature as a determinant in the distribution of marine fishes along the Pacific Coast of North America. His analysis of the changes in fauna with changes in ocean temperatures forces a consideration of how temperature affects the sardine population. Walford and Mosher, in their study of the age of adult sardines by scales and the effect of environment on first year's growth, correlated deviations from the temperature norm with anomalies in the calculated first year's growth. They pointed out that the northwest winds prevailing along the coast of California during spring and summer produce upwelling of colder waters, which bring nutrients to the surface, thus making them available to the plankton on which sardines feed. Yearly deviations from normal wind force were found to be correlated positively with anomalies in the first year's growth of the sardine. In general, the correlations indicated that growth during the first year is favored by a sustained presence of diatoms at optimum abundance which, in turn, is favored by upwelling that brings low temperature and high surface salinity.

In 1939 a striking inconsistency occurred. There was a lack of correlation between growth, on the one hand, and diatom persistence and surface salinity, on the other, as well as between surface temperature and surface salinity. Although the surface water at La Jolla was abnormally warm in 1939, it was also abnormally saline. The diatom count was high, but growth of 1939 fish was subnormal. The explanation seems to be that oceanographic conditions were exceptional, resulting in a northward extension of environmental influences favoring spawning. In that year sardine eggs and larvae were collected as far north as the Oregon coast, where growth is slower. Thus, the northern extension of the range of the 1939 year class may account for its abnormally low first year's growth in spite of the apparently favorable food supply in Southern California.

I am reminded of a parallel instance in the Bay of Fundy during 1930, when I was studying the growth of *Mya arenaria*. Studies of the effects of temperature and diatom abundance on the growth of the soft-shelled clam had indicated that growth usually reached a standstill by November. However, in 1930, it continued into December. The explanation seemed to lie in a northward extension of oceanographic conditions that normally stopped at Cape Cod, with the result that an unseasonal diatom population prevailed throughout the fall season and into early winter, as shown by the work of Viola Davidson. Hence, it seemed reasonable to believe that the food factor accounted for the unusually late growth.

Clearly, the formation of the annual ring of the *Mya* shell and the sardine scale ring is determined by a delicate, but nonetheless real, balance. The usefulness of this biological ring in commercial fishery investigations is well known. It represents one scientific contribution that has received wide acceptance by the nonscientist in the field of administration. In fact, during the past ten years there has been copious evidence of change from the one-time aversion of administrators to biological interpretations. Respect for scientific facts has manifestly grown in recent years, and I think the fish industry has gained by the costly lesson of the sardine episode. Certainly, the contributions of the California biologists are no longer in danger of frontal assault or even skeptical indifference. Concrete, incontrovertible observations and experimental evidence are insured against repudiation, whereas theoretical abstractions fail to convince. To effect closer cooperation among industry, administration, and science, effort should be directed toward more effective organization of research facilities. Such organization is now taking place, thanks to large governmental expenditures. We find ourselves thinking in terms of complex population problems, reducing biotic community interactions to mathematical expressions; and even more encouraging is the fact that industry, in not-too-isolated instances, is using some of the very same terms. This is a sign of progress. Committee reports to the Congress are, I believe, progressively more technical and, I might say, convincing. Witness the new annual appropriation of about one million dollars for Pacific Ocean tuna investigations. This marks the beginning of a new era in fishery research, made possible because biologists are at last concerning themselves with the practical problem of production.

The time for this realistic approach is long overdue. Natural populations on land and in the sea are fast disappearing. We seek a normal population, a normal biotic community, a normal factorial pattern as a base for understanding population balances. Applied biology may furnish an explanation for years of plenty, as well as for years of scarcity. But what is plenty? Too often a year of profitable catch is considered to be a year of plenty. To the sardine industry, 1936–37 was a year of plenty. But we know now that catch per unit of fishing effort, rather than total take, is the critical index of abundance.

I have referred with some optimism to the gains registered in the field of scientific fishery management within the past decade. Perhaps the primary reason for encouragement is the progress toward a particular goal, namely, teamwork. It is working in the sardine industry. It can be made to work in other fishing industries. No greater proof of the imperative need for teamwork in scientific affairs is the wastage of our heritage in natural resources by that monstrous spec-

tacle, soil erosion. Once scarcely associated with fish production, today its prevention is the hope of thousands of fishermen; in fact, the prevention of soil erosion could be their salvation.

In concluding these remarks, I wish to emphasize the role played today by applied ecology in sport and commercial fisheries. We meet it on every hand. The primary problems in fresh-water fisheries today concern interrelations between species, and population balance. In the ocean, interest centers on the effect environment exerts on population numbers. If, through careful study, the uncertainties can be eliminated, at least in large part, then the road is clear to measure the influence of the fishery on the maintenance of a high economic level of production.

All this investigational subject matter goes by a variety of names, but I can think of none more appropriate than *applied ecology*. The time has come to crystallize our thinking in regard to these particular categories of knowledge, to encourage a uniform terminology, and to utilize an old and well-established concept—namely, the application of science to human affairs.

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Technical Papers

Ultracentrifugal Studies of γ-Globulins Prepared by Electrophoresis-Convection

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The development in recent years of chemical and physical methods for the fractionation of human and animal blood plasma and serum has made possible the separation of certain components in a state of high purity and others in varying degrees of purity. The physical chemical characterization of the various plasma proteins is important from both a theoretical and a practical point of view. First, it yields information as to the molecular characteristics of these materials, thus giving insight into their reactions and physiological activity and an understanding of those properties that must be employed in the further development of fractionation procedures. It is also indispensable in following the fractionation and assuring uniformity of the products. Electrophoretic analysis has proved well suited as a criterion of purity, and ultracentrifuge studies have been most useful in detecting subtle changes in protein fractions both during preparation and during storage.

Of particular interest to the immunologist and immunochemist is γ-globulin, since this electrophoretic component of serum contains antibodies against a variety of pathogenic agents and other antigens. A number of ultracentrifugal studies of human γ-globulin, prepared by the low-temperature ethanol fractionation procedure, have been reported. In 1944 Williams and his co-workers (*1*) reported studies on 35 preparations of Fraction II of plasma. Electrophoretically these materials analyzed about 87%, and in some cases to 95%, γ-globulin, the main impurities being β-globulin and albumin. Ultracentrifugal studies of these preparations revealed about 5% slow-moving component with sedimentation constant of $S = 4$–5 Svedberg units, 75% normal component with $S = 7$, and 20% fast-moving component with $S = 8$–18. The slow-moving component presumably represented albumin, at least in part. The amount of fast-moving component, in large part γ-globulin, was quite uniform in nearly all preparations. Since that time Deutsch and his co-workers (*2–4*) have studied a number of γ-globulin fractions with different electrophoretic mobilities and purities of 95–99% as judged by electroproresis. In the ultracentrifuge at least 3 components with average sedimentation constants of $S = 7$, $S = 8$–12, and $S = 18$–20 are discernible. It has been found that as the mobility of these fractions increases from -0.97×10^{-5} cm^2 sec^{-1} v^{-1} to -2.63×10^{-5} at pH 8.6, the percentage of $S = 7$ component decreases from about 89 to 57%, with a corresponding increase in the heavier components. Nichol and Deutsch (*5*) have reported that 93% of one preparation of γ-globulin sedimented with $S = 7$.

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[2] Contribution No. 1,514.

TABLE 1

CHARACTERIZATION OF γ-GLOBULIN FRACTIONS

Source	Electrophoretic analysis		Ultracentrifugal analysis
	Percentage γ-globulin	Mean mobility $-10^5 \times \mu$ of the γ-globulin	
(a) Prepared by electrophoresis-convection			
Normal human serum from single donor stored 10 months at 3° C	98	1.32	99% S = 6 1% S = 18
Normal human serum from single donor 2 days old	> 98	1.25	99% S = 6 1% S = 18
High-titer, human Rh antiserum 1 week old	> 98	0.92	99% S = 6 1% S = 18
Pooled human serum from 150 donors 4 days old	96	1.38	95% S = 6 2.5% S = 8 2.5% S = 18
Bovine serum from single slaughterhouse steer 4 days after collection	95	1.28	99% S = 6 1% S = 18
Bovine serum from single slaughterhouse steer 1 week after collection	82	1.48	99% S = 6 1% S = 18
Pooled, high-titer, anti-RBG rabbit serum stored several months in deep freeze	> 98	1.18	> 99% S = 6
(b) Prepared by ethanol fractionation			
Armour's Fraction II	> 98	1.47	93% S = 6 7% S = 8
Fraction G obtained from Armour's Fraction II	> 98	2.25	75% S = 6.5 25% S = 8–12

Recently Smith and D. M. Brown (*6*) have reported studies of the sedimentation behavior of bovine γ_1- and γ_2-globulin, prepared by ethanol fractionation, and two fractions of γ-globulin separated by electrophoresis-convection from Armour's Fraction II of bovine plasma, also prepared by ethanol fractionation. In each instance, the main component, 80–90% of the total sedimenting material, had a sedimentation constant of about $S = 7$, and was always associated with a second component of about $S = 10$. These workers also found that equine γ- and T-globulins containing high tetanus antitoxic activity likewise contain components that sediment at about 7 S and 10 S. Components with larger sedimentation constants were not detected in these preparations.

On the basis of preliminary studies by Savat (*7*), which indicate that electrophoretically separated γ-globulin of various species shows little or none of the $S = 8$–12 component, Cohn, Deutsch, and Wetter (*2*) conclude that there is an uncertainty as to whether this component of γ-globulin is a new component formed during the chemical fractionation procedure. In view of this it appeared desirable to carry out ultracentrifugal studies on γ-globulins separated from the sera of various species by electrophoresis-convection. This method of fractionation of proteins in solution is a mild physical one that achieves separation of the components of a protein mixture by making use of small differences in their mobilities and isoelectric points. The details of construction and operation of the electrophoresis-convection apparatus and its application to the fractionation of the serum proteins have been described previously (*8*).

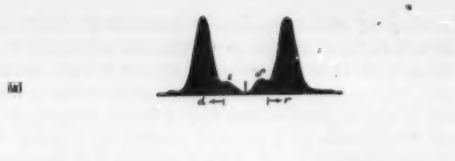
γ-Globulin was separated from normal human and bovine sera by fractionation at pH 7.0. Fractions of γ-globulin were obtained from a high-titer, anti-Rh human serum and pooled, high-titer, anti-RBG rabbit serum[3] by fractionation at pH 8.1 and 7.5, respectively. Fractionations were carried out in phosphate buffer, ionic strength 0.1, for 48 hr at field strengths of 1.5–2 v/cm. After removal from the electrophoresis-convection apparatus, solutions of the normal human and bovine γ-globulins were dialyzed against Na_2HPO_4 solutions, ionic strength 0.05, for 24 hr at 3° C, and then dried by lyophilization. Samples for electrophoretic analysis were dissolved in distilled water and equilibrated against buffer at 3° C. The solutions of the γ-globulin fractions from the anti-Rh serum and the rabbit antiserum were concentrated by pervaporation. Portions were then equilibrated against either buffer or saline solutions at 3° C.

Electrophoretic experiments were carried out on 0.8–1% protein solutions in barbital buffer, pH 8.6 and ionic strength 0.1, at a field strength of 8 v/cm for 2 hr. The results of the electrophoretic analyses are presented in Table 1. The preparations analyzed from 82% to more than 98% γ-globulin, the principal impurity being β-globulin.

The ultracentrifuge used in these studies is a synchronous-motor, direct-drive instrument designed and built in the Chemistry Department of this institute. A detailed description of it will appear elsewhere. Runs were performed at 53,160 rpm at 21°–22° C on 0.8–1% protein solutions. Sedimentation was followed by the usual Philpot-Schlieren optical method. The sedimentation constants reported in this paper are those determined in the particular solutions studied, and are not values extrapolated to zero concentration. The human and bovine γ-globulins were studied in barbital buffer, pH 8.7 and ionic strength 0.1; and the rabbit γ-globulin fraction in 0.15 *M* NaCl. Several representative sedimentation patterns are shown in Figs. 1, 2, and 3.

The electrophoretic and sedimentation patterns of a γ-globulin preparation from normal human serum from a single donor are shown in Fig. 1. In the case of the γ-globulin preparations from human sera obtained from single donors, about 99% of the material sedimented as a single component, with a sedimenta-

[3] Serum of rabbits immunized against bovine γ-globulin-*p*-azophenylarsonic acid. *R* refers to the azophenylarsonic acid and *BG* to the bovine γ-globulin. This serum and the anti-bovine serum albumin rabbit serum used in this study were kindly supplied by Dan Campbell.



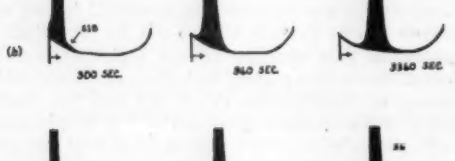


FIG. 1. (*a*) Electrophoretic pattern of γ-globulin of normal human serum, individual donor; (*b*) sedimentation patterns of γ-globulin of normal human serum, individual donor; (*c*) sedimentation patterns of γ-globulin of pooled, normal human serum, about 150 donors.

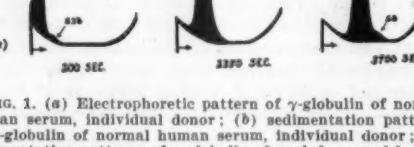


FIG. 2. Sedimentation patterns of (*a*) Fraction G of Armour's Fraction II, Sample A, 3,100 sec; (*b*) Armour's Fraction II, Sample B, 3,180 sec; (*c*) γ-globulin separated from normal bovine serum, individual slaughterhouse steer, by electrophoresis-convection.

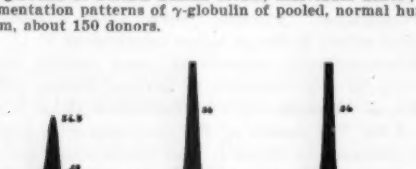


FIG. 3. Sedimentation patterns of a fraction of γ-globulin separated from pooled, high-titer, anti-RBG rabbit serum by electrophoresis-convection.

tion constant of about $S = 6$. The trace component had a sedimentation constant of about $S = 18$. In the case of the γ-globulin of the pooled normal human serum, 95% sedimented as a single component with $S = 6$, 2.5% as a component with $S = 8$, and 2.5% as a component of $S = 18$.

The bovine γ-globulins prepared by electrophoresis-convection analyzed greater than 99% of a single component with $S = 6$, the trace component having a sedimentation constant of $S = 18$. As control experiments, a sample of Armour's Fraction II of bovine plasma, prepared by ethanol fractionation, and Fraction G of bovine γ-globulin were studied. The Armour's Fraction II analyzed greater than 98% γ-globulin, with mean mobility -1.47×10^{-5} at pH 8.7. Fraction G, with mean mobility of -2.25×10^{-5}, had been previously separated by electrophoresis-convection from another sample of Armour's Fraction II (*8*), and was one of the fractions studied by Smith and Brown (*6*). Ultracentrifugal studies of Armour's Fraction II and Fraction G were carried out in barbital buffer and 0.1 *M* NaCl, pH 6.5, respectively. The Armour preparation analyzed 93% of $S = 6$ component and 7% of $S = 8$ component; Fraction G, 75% of $S = 6.5$ and 25% of $S = 8$–12. Sedimentation patterns of Armour's Fraction II, Fraction G, and γ-globulin prepared from normal bovine serum by electrophoresis-convection are compared in Fig. 2.

To within the limitations of the resolving power of the optical system, the fraction of γ-globulin from high-titer, anti-RBG rabbit serum sedimented as a single component with sedimentation constant of $S = 6$. This result is particularly interesting in that this fraction was 50% precipitable by the R-antigen. Thus, the anti-R bodies sediment at the same rate as normal γ-globulin. Campbell and co-workers (*9*) have found a light-scattering molecular weight of 158,000 ± 10,000 for purified anti-R body prepared by dissociation of specific precipitates. This is the accepted value for normal rabbit γ-globulin. Several samples of γ-globulin prepared by $(NH_4)_2SO_4$ fractionation of pooled, high-titer antibovine serum albumin rabbit serum were also studied. These materials were about 20% precipitable by bovine serum albumin. Ultracentrifugal analyses of these materials, carried out in phosphate buffer, pH 7.6 and ionic strength 0.1, showed about 99% of one component, with a sedimentation constant of about $S = 6$ and about 1% of a component with $S = 18$. Nichol and Deutsch (*5*) have studied rabbit γ-globulin prepared by ethanol fractionation. Ninety-five per cent of their preparation sedimented as a component with sedimentation constant of $S = 7$.

The results presented here are in contrast to the results of sedimentation studies on γ-globulins prepared by ethanol fractionation. Except for the case of the γ-globulin derived from the pooled human serum, which contained about 5% of material with $S = 8$–20, an amount much smaller than is usually found with ethanol fractionated samples, all the γ-globulins prepared by fractionation by electrophoresis-convection were about 99% one component. We have thus shown that this latter method of fractionation does not produce components with $S = 8$–20, and have eliminated Seitz filtering, lyophilization, pervaporation, and prolonged storage of the sera either at 3° C or in the deep freeze as causes of their appearance. Although it seems likely that the $S = 8$–20 components

of γ-globulins prepared by ethanol fractionation are new components formed either during the fractionation procedure itself or during manipulation of the fractions, the possibility cannot be excluded on the basis of our results that the $S = 8$–20 components either appear in connection with the pooling of the individual sera or are present in some, although not all, individual sera.

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Unusual Fracture Traverse in Fluorite

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For the past several years the microscope technique fractography has been studied in the author's laboratory, particularly under sponsorship of the Office of Naval Research, and, in the course of investigating the fractographic technique as a possible tool in mineralogy (*1*), some interesting and informative patterns were observed. One of these, for fluorite, warrants particular mention.

In Fig. 1 a cleavage traverse appears that is typical of patterns for good crystalline specimens of fluorite. This particular sample was a single crystal provided by N. W. Buerger, of the U. S. Naval Postgraduate School in Annapolis, Md. Cleavage, as is customary, has followed octahedral planes to provide the photographed facet, and an intersecting cleavage profile crosses the field. Instead of fracturing on a single plane, however, the separation has visibly progressed along a succession of parallel lamellae, producing a tearline structure of rather regular features. The angularity of the markings suggests a relationship of the tearline phenomenon to crystal structure, and close observation of the fractograph will disclose a fine structure both as superficial striae and as a minute stepwise progression of the tearlines. These suggest a subtle imperfection structure of regular type, such as that postulated by the micellar theory (*2*), but the pattern is otherwise conventional.

In Fig. 2, however, an elaborate structure is displayed, one that does not submit readily to conventional theories for crystal structure and fractological behavior of the solid state. The specimen is polycrystalline fluorite from Ferry County in the state of Washington, submitted by Grant M. Valentine, of the Department of Conservation and Development. The

Fig. 1. Fractograph of octahedral cleavage in fluorite, revealing crystallographic features and suggesting a lamellated structure of the crystal. (×175.)

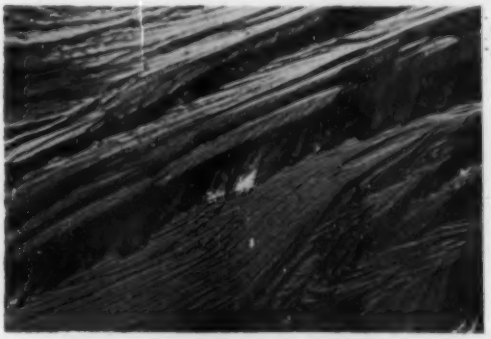

Fig. 2. Unusual fracture traverse in a fluorite crystal, showing an elaborate pattern of unsolved origin. (×237.)

field of the fractograph lies within the boundaries of a single grain. Accordingly, because the entire field has a common molecular orientation, the pattern seems clearly to indicate either (*a*) growth imperfection of the lineage or dendrite type, or (*b*) complex stress-strain relationships whose cause is unknown.

If it is a matter of growth imperfection, the over-all molecular structure can be understood to have achieved an approximately uniform orientation at the time of the original growth of the crystal; but throughout that growth there became distributed the observed elaborate pattern of imperfection. The micellar theory (*3*) accounts for such patterns on the basis of a deposition of molecular clusters, or micelles, from the liquid state. The molecular structures of the individual micelles maintain the approximate orientation common to the field, thereby constituting the defined monocrystal, but a displacement is suffered among the micelles themselves. Fracture, influenced by paths of minimum cohesion, tends strongly to proceed along the intermicellar and interlineage boundaries, thereby depicting the imperfection architecture, of which the fractograph in Fig. 2 may be an excellent example.

If the pattern is not directly referent to historical features of crystal formation, it must then express

complex configurations in the transient rupturing stress, such as those illustrated by Kies, Sullivan, and Irwin (*4*).

In either case, the fracture traverse exhibits marked characteristics, the interpretation of which must lead to important information concerning the nature of cohesion and dehesion in the solid state.

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Density of Polystyrene Latex by a Centrifugal Method

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Particulate densities in solution or the reciprocal of the partial specific volume have been determined by a number of investigators by centrifugal means. The method used has involved the determination of the density of solvent for which the velocity of sedimentation of the particles is zero. This was done either by extrapolation of the velocity-density relation from low solvent densities to the iso-density point, by interpolation between low and high densities of solvent, or by experimentally adjusting the density of the solvent to the point at which no sedimentation occurred.

For a review of the work prior to 1942 the reader is referred to the article by Markham, Smith, and Lea (*1*). Similar investigations have since been reported (*2, 3*).

The idea occurred to us of arranging a density gradient in the centrifuge tube in such a manner that the sedimenting particles would concentrate in the central part of the tube at the position where the densities of the particles and solvent were equal. This would be accomplished by establishing a constant density gradient so that in the upper part of the tube the particles would be more dense than the solvent, whereas in the lower part of the tube the particles would be less dense than the solvent. A constant density gradient (linear density scale) between the extremities of the solution would permit by a simple distance measurement the determination of the density at any point in the tube, the density of solvent at the concentration band being the density of the particles.

As a trial material to test the method, polystyrene latex, lot 580G, Dow Chemical Company, was chosen. This has been reported to have a density of 1.052 (*4*),[1] 1.053 (*5*), and 1.054 (*6*).

For centrifugation, the standard 35° angle rotor holding 5-ml tubes and a swinging tube rotor (*7*) holding 1.5-ml tubes were used. The latex, .01% to .03%, was suspended in 5 solutions of graded density, first calculated from known concentrations and then checked by pycnometer measurements. The solvents used were sucrose and heavy water with 1% NaCl added. After centrifuging for 2–4 hr, depending on the gradient in density, the tubes were removed from the rotor and the position of the concentration band was measured. In some cases they were photographed using side illumination to obtain scattering with little direct transmission (Fig. 1).

[1] This value was obtained for material in the dry state.

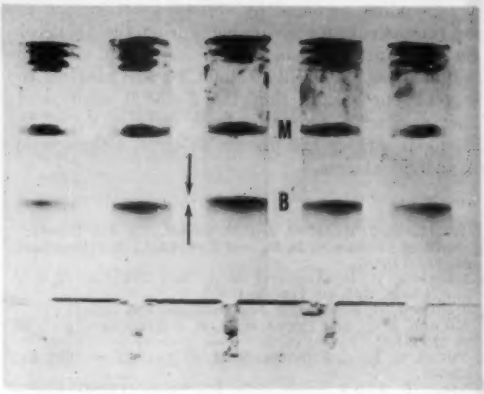

Fig. 1. Five tubes removed from angle rotor and mounted in holder. Tubes contain 5 ml .01% latex in sucrose + 1% NaCl, densities ranging from 1.030 at top to 1.099 at bottom of tubes; centrifuged 2 hr at 500 rps. Position of meniscus at *M* concentrated band of latex at *B* and apparent direction of sedimentation indicated by arrows.

In the swinging tube rotor 6 tubes with heavy water and NaCl as solvent were used, and in the angle rotor 14 tubes were used with sucrose and NaCl. The greatest density range used was 1.030–1.099, and the smallest was 1.052–1.061; the most satisfactory was found to be 1.048–1.065. With too large a range the concentration band is narrow and sharp, but the accuracy is small because a small error in the determination of the band position introduces a large error in the density. With too low a gradient the band is broad and diffuse, and the time of centrifugation must be unduly prolonged.

In the case where the iso-density point of the solvent extends over several millimeters a double band builds up. Each edge of the constant density region acts as a barrier for the sedimenting material, giving a high concentration at the edges of the constant density region and the original concentration in the center of the band, since no motion occurs within the region. The measurement under these conditions is made from the meniscus to the center of the double band.

The value obtained from six runs was 1.055 ± .001. No significant difference could be detected between sucrose and heavy water as solvents. The concentration of latex in the central band was of the order of ten times the original concentration.

The gradient density method is simple and rapid and is not appreciably affected by viscosity or other

hydrodynamic factors. There was no significant difference between sucrose and heavy water as solvents, and since Sharp and Beard (*5*) found the same density in heavy water and albumin, it follows that the density is the same, within experimental error, in all three solvents. The same densities were obtained in the angle and swinging tube rotor. The value obtained for the density of the latex sample used, 1.055, is in fair agreement with the reported values of 1.052, 1.053, and 1.054 obtained by other methods.

The density gradient method should, when applied to purification problems, lead to a separation of particles having different densities, irrespective of their size.

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Prolongation of Thiopental Anesthesia in the Mouse by Premedication with Tetraethylthiuram Disulfide ("Antabuse")[1]

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It has been our purpose in this work to investigate certain substances, commonly encountered in clinical practice or occurring naturally, that are potentially capable of prolonging the anesthesia produced by thiopental. Brodie *et al.* (*1*) have shown that thiopental undergoes incomplete side-chain oxidation to a carboxylic acid in its metabolism by the normal mammal. The compounds that fell in our sphere of interest, therefore, have been those that might interfere with such oxidation. Reducing agents such as ascorbic acid and cysteine have, in fact, been found to potentiate thiopental anesthesia, presumably through this mechanism (*2*).

Tetraethylthiuram disulfide ("Antabuse") has achieved some clinical usefulness in the treatment of chronic alcoholism, probably by virtue of its blocking of the oxidation of ethyl alcohol at the acetaldehyde stage (*3*). It has been shown, further, that this inhibition by Antabuse is antagonized by "Ferroascorbin" solution[3] (*4*). The recent report by Richert and co-workers (*5*) that Antabuse can inhibit rat liver xanthine oxidase stimulated our interest in this compound. It seemed possible that Antabuse might prolong thiopental anesthesia through this inhibition. Therefore, the effect of Antabuse on thiopental anesthesia in the mouse, and the influence of Ferroascorbin on this interaction, were studied.

TABLE 1

Influence of Antabuse on Thiopental Anesthesia in the Mouse and Failure of Ferroascorbin to Inhibit this Influence

Group	No. animals	Mean duration of anesthesia (min)
1 Thiopental control	20	4.7
2 Antabuse plus thiopental	10	256.2
3 Antabuse plus thiopental plus Ferroascorbin	5	221.5

Three groups of adult male albino mice (Carworth Farms), weighing 23–25 g, were selected at random. The anesthesia time in all three groups was determined for 30 mg thiopental/kg given intravenously. The criterion for recovery from anesthesia was the return of the righting reflex. The three groups were given the following medication prior to the determination of the anesthesia time:

Group 1: No premedication.

Group 2: Premedication with Antabuse.[4] Each animal received a daily oral (by intubation) dose of 25 mg Antabuse for 3 such doses.

Group 3: Premedication with Antabuse, and postmedication with Ferroascorbin. Each animal received Antabuse as in Group 2. On the third day, the injection of thiopental was immediately followed by 0.1 ml Ferroascorbin solution given intravenously to each animal.

The results of these experiments are summarized in Table 1.

This sixtyfold increase in thiopental anesthesia time produced by Antabuse is by far the most powerful effect of this type observed by us in our studies. Determinations of anesthesia time in Group 2 at random during the premedication period revealed no prolongation until after 3 days of the premedication period had elapsed. The ferrous iron and ascorbic acid solution, it may be observed, failed to prevent the Antabuse effect to any significant extent. A 2-week postanesthetic period of observation revealed no toxic abnormalities in the behavior and growth of the experimental animals.

Richert's analysis of his data (*3*) is of especial interest in the light of this work. He has concluded that Antabuse inhibits the oxidase action of xanthine oxidase, which action is responsible for reoxidation of the reduced enzyme by atmospheric oxygen. Appar-

[1] This work has been supported by the James Hudson Brown Fund of Yale University Medical School.

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[3] "Ferroascorbin" solution contained 27.5% $FeSO_4 \cdot 7H_2O$ and 1.25% ascorbic acid in distilled water.

[4] We are indebted to C. E. Glud, formerly of the Department of Pediatrics, Yale University School of Medicine, for supplying us with this material.

ently the dehydrogenase action of xanthine oxidase is not interfered with by Antabuse. In view of these facts, in order for Antabuse to have an effect in the whole organism one might expect some time to elapse in order to allow all the dehydrogenase function to be reduced, before any true inhibition may be observed. This was well borne out by our studies, since we found that a period of 3 days of Antabuse dosing was necessary before the thiopental potentiation became evident. It might be argued that the 3 days were necessary because of the cumulative dosage, but this hardly seems pertinent since the individual doses were massive, the 25 mg/mouse representing about 1,250 mg/kg.

Finally, these data lead to the inference that xanthine oxidase is one enzyme functioning in the metabolism of thiopental to thiopental carboxylic acid. Further work designed to substantiate this inference will be reported later.

It is concluded that Antabuse, possibly through its inhibitory effect on xanthine oxidase, has the ability to prolong markedly the anesthesia induced in the mouse by intravenous thiopental. Pending investigations in higher mammals, it is suggested that thiopental be administered with caution to individuals who are undergoing treatment with Antabuse.

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Infrared Microspectrum of Living Muscle Cells[1]

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The absorption spectra of many animal and plant tissues have been reported in the literature. For the ultraviolet, several authors (*1–3*) have reported successful microspectra of tissue cells and parts of cells, both living and fixed. For the infrared the macrospectra of dried tissues (*4, 5*) and the microspectra of individual dried cells (*6*) have been reported. Preliminary spectroscopic measurements on living muscle cells in the wavelength region from 1 to 15 μ were undertaken by the author to establish the feasibility of using infrared spectroscopy as a tool in studies of living tissues. These measurements are still incomplete, but the data so far collected are presented to show that the difficulties recently reported (*7*) in measuring useful microspectra of living tissues are not insurmountable.

[1] Part of this work was done under an AEC predoctoral fellowship in the Biological Sciences at Ohio State University.

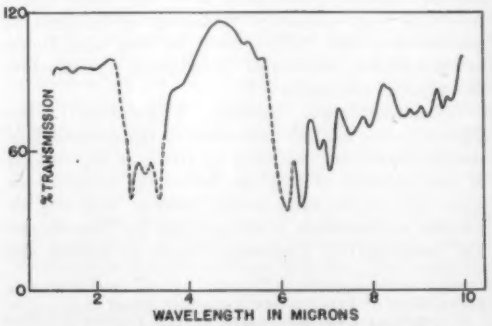
[2] USPHS postdoctoral fellow, National Institutes of Health.

It seemed desirable to use the individual cellular unit of muscle for this work, both because of the difficulty of preparing suitable specimens in any other form, and because in some cases a variation is known to exist in the nature and function of different cells from the same muscle. For successful spectroscopy of such small specimens (20 μ–180 μ diam) in the infrared, it was necessary to use the microspectrometer described elsewhere (*8*), which was originally designed for studies of small fibers.

The specimens were prepared as follows: From the freshly isolated muscle a fiber was teased out under Ringer's solution. The isolated fiber was mounted in Ringer's between transparent plates (fluorite or silver chloride) and placed in the radiation beam of the microspectrometer. The spectrum was then recorded. If the fiber was too small to give a satisfactory spectrum, from 1 to 4 fibers were laid side by side to form a larger absorbing area. The spectra were considered to be representative of the living tissue if the fiber (or fibers) could be made to contract visibly on electrical stimulation after the spectrum had been measured. It was not always possible to measure the whole spectrum before the contractility of the fiber was lost. The interesting region from 6.5 to 10 μ could be examined conveniently in two sections, although it was possible in some cases to get through the whole region without losing contractility.

The principal difficulty in measuring the infrared spectrum of any living tissue is the large ratio of water to protoplasm. It is well known that water is a very strong absorber of infrared radiation. As a result, the absorption bands of the muscle are nearly obliterated by the very intense absorption of the water. The difficulty was resolved in this case by using a spectrometer of maximum sensitivity and measuring the absorption of the cells relative to the absorption of the aqueous suspension medium. The ratio of energy transmitted through the cell to that transmitted through the same thickness of Ringer's solution next to the cell was plotted against wavelength to obtain the spectrum.

The spectrum from 1 to 10 μ for a fiber from the gastrocnemius of a frog is shown in Fig. 1. It was



SPECTRUM OF A MUSCLE CELL FROM THE FROG GASTROCNEMIUS

Fig. 1.

found that beyond 10 μ the absorption of the muscle fiber in the preparations studied was identical with that of an equal thickness of Ringer's solution. In the region from 6.5 to 10 μ there are several strong and sharp absorption bands (to be discussed later). In the region from 5.7 to 6.5 μ the absorption of the muscle is obscured by the strong band of liquid water in that region. At wavelengths shorter than 6 μ the spectrum has not been intensively studied. The very interesting 3-μ region is almost completely obscured by the strong water band at that wavelength, and between 3.5 μ and 6 μ the absorption bands of the preparations used were weak and diffuse. The dotted portions of the curve were drawn to represent the spectrum of the tissue that one would expect on the basis of the dehydrated material. The region from 6.5 to 10 μ seems to be the most useful portion, according to present information.

In this region the eight bands listed in Table 1, with their wavelengths and frequencies, occurred with enough intensity and regularity to be considered real. The bands not tabulated have not been studied extensively as yet.

TABLE 1

Band designation (Fig. 2)	Wavelength (μ)	Frequency (cm^{-1})
a	6.50	1,538
b	6.85	1,460
c	7.10	1,408
d	7.65	1,307
e	8.00	1,250
f	8.5 –8.7	1,175–1,150
g	9.2	1,087
h	9.7	1,031

It was found that, although many of the bands were present in the spectra of all the muscle tissues studied, there were some differences among the various fibers. The bands marked *a–g* in Fig. 2 (curve *F*) occurred in all muscle spectra, as well as that of the extracted protein myosin (curve *G*, Fig. 2). However, the band marked *h* in Fig. 2 (curve *F*), falling at 9.7 μ, was not found in all the spectra, as can be seen by comparing curves *A, B, D, E, F* in Fig. 2. The band was strongest in the frog gastrocnemius fibers, and in turtle ventricular muscle. It was totally absent, or extremely weak, in frog ventricular muscle, frog sternohyoideus muscle, and many others. The occurrence of the band is tabulated in Table 2, with the minus signs indicating the absence of the band, and the number of plus signs indicating roughly the intensity of the band if it did occur.

Every entry in Table 2 represents at least two different spectra, each necessarily of different fibers, and each usually from a different muscle. A total of 77 spectra is represented. No correlation has been observed between the presence or absence of this band and any physiological or structural differences in the muscles.

Other differences appearing in the spectra, such as

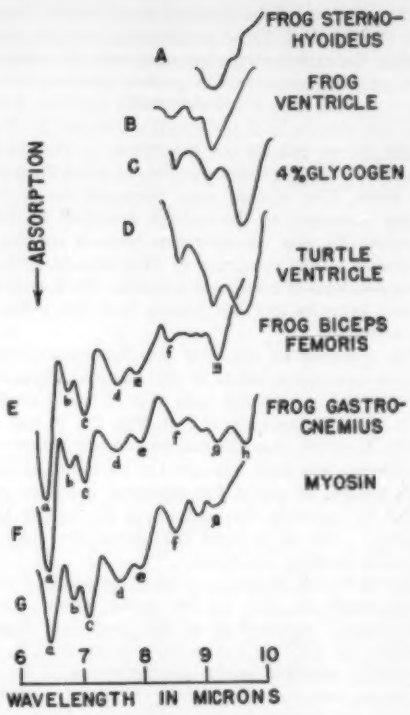


FIG. 2.

the presence of the small band between those marked *f* and *g* in Fig. 2 (curve *F*) have not been studied well enough at present to be discussed here. Further work is in progress.

In addition to the muscle spectra themselves, the

TABLE 2

Animal	Muscle	Band at 9.7 μ
Frog	Tibialis anticus	++
"	Sartorius	++
"	Gastrocnemius	++
"	Sternohyoideus	–
"	Hyoglossus	–
"	Ventricle	–
"	Biceps femoris	–
Turtle	Ventricle	+++
"	Auricle	+++
"	Rectus abdominis	++
"	Pectoralis	±
"	Neck, retractor	+
"	Trapezius	± (weak)
"	Extensor digitorum	–
"	Mixed red and white	±
Cockroach	Wing	–
"	Leg	–
Rabbit	White	–
"	Red	–

spectra of three of the chemical constituents of muscle have been studied. These constituents were the protein myosin, the carbohydrate glycogen, and the nucleotide adenosine triphosphate. The protein spectrum was not easy to obtain from the undenatured material. Indeed, it is not certain that the spectrum shown in Fig. 2 (curve *G*) represents the absorption in the undenatured state, although the spectrum of dried films is not the same. The myosin was prepared from rabbit muscle according to the method described by Szent-Györgyi (*9*), and the spectrum bears a striking resemblance to the spectrum of frog muscle, although correspondence is surely not complete. More studies of protein extracts and the muscle used for extraction are indicated.

The spectrum of glycogen was very interesting in that an aqueous solution of the material showed absorption almost identical with that of turtle ventricular tissue (*cf.* curves *C* and *D*, Fig. 2). It was concluded, however, that the correspondence between the two spectra was only coincidental, on the basis that a concentration of about 4% glycogen in water is required to duplicate the contour of the muscle tissue spectrum. This is at least four times the maximum normally found in the tissue.

The studies on adenosine triphosphate gave discouraging results in view of the great interest in the physiological significance of this material. Aqueous solutions of ATP in concentrations up to 1% gave completely negative results spectroscopically. In fact, no success was encountered in making water solutions concentrated enough to show absorption bands in the region under consideration, although the solid material absorbs rather strongly. The implication of this result is, of course, that none of the other substances present in small amounts (less than 1%) in muscle can be studied by this technique unless their molar extinction coefficients are considerably larger than that of the ATP. If this is true, the usefulness of the technique will be greatly limited since all but the protein components of muscle will not be detected. Indications are, however, that the protein fraction of the living tissue can be studied through its infrared spectrum. This opens up a large field of study, even though enzyme systems and many metabolic processes are below the threshold of detection.

The results of this preliminary investigation may be stated as follows:

1. It is feasible to measure a useful absorption spectrum of living muscle cells in the infrared region of the spectrum. There appears to be no reason why other living cells will not also yield useful spectra.

2. The useful region of the spectrum of the living tissue is limited to that between 6.5 and 10 μ by the large ratio of water to protoplasm.

3. The spectrum of muscle from 6.5 to 10 μ wavelength shows sharp and strong bands, not all of which occur with regularity in the tissues studied. The absorption band at 9.7 μ is particularly interesting in comparative studies because it appears so strongly in some cells and not at all in others that are physiologically similar.

4. The spectrum of the muscle cell seems to be closely duplicated by that of the protein myosin, although it is certain that other as yet unidentified substances are contributing strongly to the absorption. The substances found in muscle in concentrations less than 1% probably do not contribute to the spectrum, ruling out many biochemical reactions as not being detectable by this technique. The proteins of muscle, however, seem to lend themselves to this method of study.

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Amino Acid Constituents of Prochromosomes Isolated from Blood Cells of Various Animals[1]

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The threadlike bodies isolated from metabolic nuclei apparently resemble in size, appearance, and microstructure the chromosome in anaphase, for which reason they were given the name prochromosomes[2] (*1*). The prochromosomes isolated from blood cells of various animals have been studied with the help of an electron microscope. Each prochromosome is composed of a pair of chromonemata free of DNA and chromatin granules in which DNA are stored (*1, 2*). The chemical constituents of prochromosomes isolated from human leucocyte and carp erythrocyte nuclei, and of salivary chromosomes of *Drosophila*, have been studied by means of paper chromatography and histochemical methods (*3–6*).

The chemical components of chromosomes are of fundamental importance and interest in genetics and cytology. For this reason paper chromatography is one of the most valuable contributions of the chemist to the study of biology. The present paper is an account of our experiments and conclusions concerning the amino acid content of the prochromosomes isolated from blood cells of various animals, chosen from the standpoint of phylogenesis. Although we believe that the present experiments advance our knowledge of the amino acid composition of the prochromosomes, it is clear that much work remains to be done before the complete chemical composition of the gene can be given.

[1] This work was supported in part by a grant from the Science Research Fund, Ministry of Education.

[2] Since this paper was submitted for publication, we have changed this term to "metabolic chromosomes."

TABLE 1

AMINO ACID CONTENT OF PROCHROMOSOMES ISOLATED FROM BLOOD CELLS IN VARIOUS ANIMALS AND OF SALIVARY CHROMOSOMES OF *Drosophila melanogaster*

Amino acid	Prochromosomes isolated from leucocyte nuclei					Prochromosomes isolated from erythrocyte nuclei					Salivary chromosomes (5)
	Man (5)	Rabbit	Guinea pig	Domestic duck	Toad	Cock	Domestic duck	Tortoise	Toad	Carp (4)	
Glycine	+	+	+	+	+	+	+	+	+	+	+
Alanine	+	+	+	+	+	+	+	+	+	+	+
Valine	+	−	+	+	+	+	+	−	+	+	+
Leucine	+	+	+	+	−	+	+	−	−	+	+
Lysine	+	+	+	+	+	+	+	+	+	+	−
Arginine	+	+	+	+	+	+	+	+	+	+	+
Aspartic acid	+	+	+	+	+	+	+	+	+	+	+
Glutamic acid	+	+	+	+	+	+	+	+	+	+	+
Phenylalanine	−	+	−	+	−	+	+	−	−	+	−
Serine	+	+	+	+	+	+	+	+	+	+	−
Threonine	+	−	−	+	−	+	+	+	−	+	−
Proline	−	−	−	−	−	−	−	−	−	−	+
Taurine (?)	+	+	+	+	+	+	+	+	+	+	−

The prochromosomes were isolated from the leucocyte nuclei of rabbit, guinea pig, domestic duck, and toad, and also from the erythrocyte nuclei of cock, domestic duck, tortoise, and toad. The nucleus membranes of blood cells of guinea pig, rabbit, tortoise, and toad were easily destroyed by means of a Waring mixer, but the membranes of cock and domestic duck were so hard that we had to resort to the mechanical action of supersonic waves and the Waring mixer, which were operated alternately to destroy the nuclei completely. The whole procedure was carried out in a cold room at about 1° C. The qualitative methods of paper chromatography and the procedure for isolating prochromosomes were the same as those already described in previous papers (*4, 5*).

In the prochromosomes dissolved in distilled water and not hydrolyzed in NaOH or HCl, no trace of amino acid has been found. Also, no tryptophane has been demonstrated in the specimens hydrolyzed with saturated barium hydroxide or 6 *N* NaOH. A complete list of the amino acids found in the present and previous experiments (*4, 5*) is contained in Table 1, which shows that practically the same amino acids are found in prochromosomes isolated from erythrocyte nuclei of both cock and domestic duck, but the same amino acids are not always found in rabbit and guinea pig. It appears, from evidence presented in this paper, that no qualitative difference is revealed in amino acid composition of the prochromosomes between the nuclei of leucocytes and erythrocytes in the same species. There are some qualitative differences of amino acid composition in the 11 preparations. But even these differences failed to clarify the phylogenetic differentiation that had been expected.

In this experiment glycine, alanine, lysine, arginine, aspartic acid, glutamic acid, serine, and taurine (?) have always been identified in all the prochromosomes isolated from the erythrocyte and leucocyte nuclei. One spot reacting to purple color with ninhydrin appears to correspond to taurine, which has been identified by R_F. However, so far as we are aware, taurine has never before been detected as a component of proteins. Whether the spot is taurine or a degradation product of other materials remains to be determined. It was considered to be "taurine" in our previous papers (*4, 5*) and in the present study. Proline has been demonstrated in the salivary chromosomes of *Drosophila* but has never been demonstrated in the prochromosomes isolated from blood cells.

In subsequent experiments we have investigated whether DNA has interfered with the appearance of tryptophane on paper chromatograms. A solution of 0.1% tryptophane in saturated barium hydroxide or 6 *N* NaOH, containing 0.1–1.0% DNA, was heated at 110° C. After 15 hr one drop of the solution was placed on the paper (Toyo, No. 2). In this experiment only the butanol-acetic acid solvent was used for identifying tryptophane, which was calculated to be 5 or 10 γ. It has been confirmed that the presence of DNA does not interfere with the reaction of tryptophane on the paper chromatogram.

The greatest uncertainty in this work lies not in the result of the analytical procedures but rather in the difficulty of getting a sufficiently pure specimen. As already shown by electron microscopy (*1, 2*), it was comparatively easy to isolate the pure prochromosomes from the metabolic nuclei of blood cells.

If there is to be a phylogenetic chemo-differentiation within the prochromosome protein components, it is probably quantitative rather than qualitative. Moreover, it apparently does not occur at the level of the amino acids, but on a higher plane of molecular organization.

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Effects of an Isolated Dehydrogenase Enzyme and Flavoprotein on the Reduction of Triphenyltetrazolium Chloride[1]

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The enzymatic reduction of triphenyltetrazolium chloride (TTC) to its red formazan has been used as a means of measuring dehydrogenase activities of biological systems (*1–6*). Bielig *et al.* (*7*), through an analysis of the pH dependence and temperature sensitivity of TTC reduction by bacterial systems, suggested the role of a flavoprotein, diaphorase, as the immediate site of the reduction. Kuhn (*8*) also has reported that TTC is reduced via the oxidation of reduced coenzyme by diaphorase. Mattson *et al.* (*9*) reported the reduction of TTC by a glucose dehydrogenase-coenzyme I system, but it was not mentioned whether flavin enzymes were involved.

We have investigated the effect of an isolated dehydrogenase on the reduction of TTC. Phosphoglyceraldehyde dehydrogenase was isolated and purified from baker's yeast as described by Meyerhof and Junowicz-Kocholaty (*10*). The activity measured was the reduction of coenzyme I (DPN) in the presence of phosphoglyceraldehyde and sodium arsenate in a borate buffer (0.3 *M* at pH 7.0). The reduction of DPN was determined by measuring the increase in absorption at 340 mμ in the Beckman spectrophotometer. When TTC (30 μg/ml) was added to this system, the rate of the reduction of DPN was not affected, nor was formazan produced, as shown by the absence of any absorption at 485 mμ (maximum peak). The millimolar extinction coefficient (485 mμ) of formazan in an acetone-water solvent at pH 6.1 was 12.4.

The oxidation of reduced DPN by diaphorase was next investigated. The diaphorase was prepared from baker's yeast by a modification of the method of Green and Dewan (*11*). When this was added to the above-mentioned system in which DPN had been reduced enzymatically, the reduced DPN was readily reoxidized as measured by decrease in absorption at 340 mμ. Under these conditions the addition of 30–250 μg TTC/ml had no effect on the reoxidation of the reduced DPN; nor was any formazan produced. However, after preliminary investigations using reduced DPN prepared by the method of Ohlmeyer (*12*), it became evident that the reduction of TTC could be accomplished by the diaphorase system under anaerobic conditions. Under aerobic conditions, however, the reduction occurred either not at all, or very poorly, over a narrow pH range. Fig. 1 shows the rate of formation of formazan at pH 7.0. Under these conditions, formazan is produced only under the anaerobic conditions obtained with conventional Thunberg tubes which had been evacuated, flushed with nitrogen, and reevacuated.

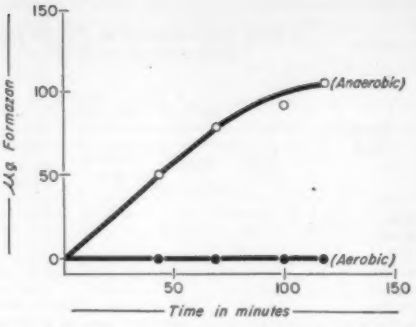

FIG. 1. Reduction of TTC by yeast diaphorase (pH 7.0). (Conditions same as in Table 1.)

It can be see from Table 1 that the reduction of TTC can occur aerobically in the narrow pH range of 7.5–8.3. The amount of formazan formed was still considerably less than was produced anaerobically under the same conditions. Under the optimal conditions (pH 8.3) of the aerobic reduction of TTC by this system, it could be demonstrated that for each mole of reduced DPN oxidized, one mole of formazan was produced (Table 2). At pH 7.0 the oxidation of the reduced DPN still occurred, though at a somewhat slower rate, and this was not affected by the presence of TTC; nor was the latter reduced.

TABLE 1

THE EFFECT OF pH ON THE REDUCTION OF TTC BY YEAST DIAPHORASE*

pH	Formazan formed after 120 min (μg/ml)	
	Anaerobic	Aerobic
5.7	0	0
6.9	150	0
7.5	350	20.5
8.0	500	50.0
8.3	650	58.0
9.2	0	0

* The system consisted of: 2.5 ml 0.5 *M* phosphate buffer (pH 6.9–8.3) or 0.2 *M* borate buffer (pH 9.2–10.0); 0.5 ml diaphorase (1/30 dilution); TTC (200 μg/ml); reduced DPN (200 μg/ml)—reduced by sodium hydrosulfite. Total system of 5 ml.

As applied to isolated systems, diaphorase has been shown to reduce TTC both aerobically and anaerobically, although the aerobic system was much less effective than the anaerobic. This may be due to (1) an elevation of the O/R potential to an unfavorable level, (2) a direct competition between oxygen and TTC for flavoprotein, or (3) the involvement of other mechanisms in the aerobic reduction of TTC. Whether

[1] This work has been aided by a grant from the Eaton Laboratories, Inc., Norwich, N. Y.

[2] We are grateful to Peter Oesper, of the Physiological Chemistry Department, for his supply of phosphoglyceraldehyde and his invaluable advice during preparation of the enzymes.

TABLE 2

OXIDATION OF REDUCED DPN AND REDUCTION OF TTC BY YEAST DIAPHORASE*

Time (min)	pH 7.0			pH 8.3		
	TTC present			TTC present		
	DPN mμM	Formazan mμM		DPN mμM	Formazan mμM	
0	0	0	0	0	0	0
5	–	9	0	–	12	12
10	9	13	0	27	21	24
20	21	24	0	54	51	39
50	53	34	0	64	58	67

* System same as in Table 1.

other mechanisms, such as the cytochrome-cytochrome oxidase systems, are involved, either directly or indirectly, is at present under investigation.[3]

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[3] Since submission of this paper, the work was continued with an isolated bacterial (*E. coli*) DPN-H_2 oxidase and TTC, as well as neotetrazolium. The findings were similar and will be the subject of a future report.

The Use and Toxicity of Pontamine Sky Blue

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The need for a better method of visualizing the primary and secondary lymphatic echelons in resectional surgery in carcinoma has long been recognized. Any agent used for this purpose should have the following characteristics: (1) high water solubility, (2) high degree of contrast compared to the tissues into which it is injected, (3) high specificity for and low diffusibility from lymph and lymph nodes, (4) low toxicity, (5) color stability, particularly near pH 7.2, and (6) ability to penetrate into the surgical area within 15 min. Braithwaite (*1*) found that indigo carmine fulfilled most of these criteria, but unfortunately the rate of dye uptake and its penetration into the surgical area left much to be desired. A review of the work of McMasters and Parsons (*2*) and Hudack and McMasters (*3*) showed that Pontamine Sky Blue (sodium salt of dimethoxydiphenyl-diazo-bis-8-amino-1-naphthol-3, 6 disulfonic acid) fitted all these criteria. In a study involving 35 cases of gastric resection for carcinoma, Weinberg and Greaney (*4*) established the utility of the dye for this purpose.

Recently the dye has been employed in more than 50 cases of intrathoracic surgery to delineate the surgical anatomy of the endothoracic lymphatics. The patient is prepared for such surgery by the usual procedure, an endotracheal catheter introduced, the thoracic cage opened and 5 ml of a 4% solution of Pontamine Sky Blue injected into the lung parenchyma near the hilum. The dye spreads rapidly, outlining the lymphatics not only in the immediate area but also in the more remote areas, thus allowing more radical surgery to be performed. This has extended the scope and completeness of extirpation of the diseased and potentially diseased tissue in bronchiogenic carcinoma. During the course of this work, 8 patients showed a residual blue coloring of the subcutaneous tissues. This was of concern not only to us but to those investigators using the dye under our directions because nothing was known about the possible toxicity of the dye.

The intravenous toxicity of Pontamine Sky Blue was determined in mice, and the LD_{50} was calculated by the Litchfield-Wilcoxon (*5*) method (Table 1).

TABLE 1

LD_{50} OF PONTAMINE SKY BLUE IN MICE

No. animals	LD_{50} g/kg	Confidence limits (odds 19/20) g/kg	Slope	Confidence limits (odds 19/20)
45	2.26	2.154–2.371	1.02	0.93–1.11

The symptoms of toxicity observed were acute respiratory embarrassment and death by cardiac and respiratory failure. The respiratory phase can be readily counteracted by artificial respiration.

Thirty animals that survived doses between 1.5 and 2.5 g/kg of the dye were observed for 28 days. Slight discoloration of the skin was observed in all the animals, but no evidence of residual toxicity was observed at necropsy.

In the use of any dyestuff, there is the possibility of traces of inorganic and organic materials being carried through the process and ending up in the final product. Such materials can produce toxic symptoms unrelated to the over-all toxic symptoms produced by the dye itself. Spectrographic analysis of Pontamine Sky Blue revealed that there were minute traces of Mn, Pb, and Cr. However, these ions were in such small quantities that they could not possibly cause any of the toxic manifestations seen when the dye was injected into humans or animals.

The possibility of toxicity caused by aging of the

4% solution used in the human studies was tested by injecting 0.5 g/kg into mice. The solution used was between 6 and 12 months of age. None of the 10 animals given the dye showed any evidence of toxicity.

When one compares the usual human dose, 5 ml 4% (0.2 g), with the LD_{50} for mice (2.26 g/kg), it is evident that there is a large safety factor involved even if the dye should inadvertently be injected intravenously. Furthermore, when one considers that most of the stained area is removed during surgery, it is probable that the total amount of residual dye is of no practical importance. Applying the toxicity criteria of Hodge and Steiner (*6*) reveals that Pontamine Sky Blue would be classed as a slightly toxic material.

A full report of the clinical utility and duration of skin staining caused by Pontamine Sky Blue will be reported in detail elsewhere.

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Ion Exchange Separation of Desoxyribonucleotides[1]

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As has been implied by Cohn (*1*), the ion exchange methods developed by him for the separation of ribonucleotides (*2*) may be readily adapted to the separation of desoxyribonucleotides. Since no procedure for this separation has been published, it would seem worth while to outline the technique developed in our laboratory.[2]

The mixed desoxyribonucleotides were prepared by a two-stage enzymatic hydrolysis of highly polymerized desoxyribonucleic acid (DRNA). Two hundred mg of DRNA, prepared from calf thymus according to the method of Mirsky and Pollister (*3*), was dissolved in 20 ml 0.5 *M* acetate buffer, pH 6.5. To this were added 10 ml 0.1 *M* $MgSO_4$ and 10 ml of a distilled water solution of crystalline pancreatic desoxyribonuclease (Worthington Biochemical), containing 400 μg/ml. The enzymatic digestion was carried out for 72 hr at 37° C, under a layer of spectroscopically purified hexane, with constant stirring by magnetic stirrer.

The second stage of enzymatic digestion was carried out according to the method of Klein (*4*), using a nuclease preparation from intestinal mucosa, in the presence of arsenate to inhibit alkaline phosphatase also present in the preparation. The pH of the digest resulting from the action of the pancreatic enzyme was adjusted to 8.5 with 2 *N* NaOH. To the digest were added 10 ml 0.36 *M* $Na_3(AsO_4)_2$, 10 ml 1 *M* $NH_4OH \cdot (NH_4)_2SO_4$ buffer, pH 8.5, and 10 ml of a solution containing 17 mg/ml of the intestinal enzyme, prepared according to Klein, as modified by Brady (*5*). Digestion was carried out for 20 hr at 37° C under hexane, with constant stirring.

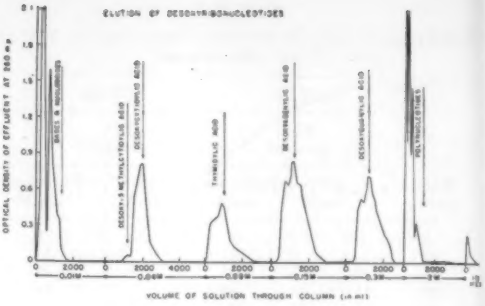

FIG. 1.

The pH of the digest was adjusted to 4.7 with glacial acetic acid to precipitate the intestinal preparation, which was then centrifuged out. The centrifugate was washed with 0.01 *M* acetate buffer, pH 4.7, and the washings were added to the previous supernatant. The pH of the combined solutions was adjusted to 9.0, and the volume made up to 250 ml.

This solution, containing mixed bases, nucleosides, nucleotides, and some undigested polynucleotides, was then added to the ion exchange column, π cm^2 × 11 cm, Dowex A-1 resin, 250–500 mesh[3] in the acetate form. Because of the marked lability of the purine desoxyribonucleotides to acid, it is desirable to employ procedures that avoid any prolonged contact with solutions of low pH. For this reason, elution from the ion exchange column was carried out in buffered solution at pH 4.3.

The elution procedure is indicated in Fig. 1. Bases and nucleosides, which accounted for 21.6% of the ultraviolet absorption of the material added to the column, were eluted with 0.01 *M* acetate buffer, pH 4.3. Desoxy-5 methyl cytidylic acid and desoxycytidylic acid were eluted, with incomplete separation, with 0.04 *M* buffer. If it is desired to separate these completely, they may be recycled on an acetate column, using buffer at pH 4.7 for elution (*1*). Thymidylic acid was removed with 0.08 *M* buffer, desoxyadenylic with 0.15 *M* buffer, and desoxyguanylic with 0.3 *M* buffer.

Subsequent passage of 2 *M* acetate buffer, pH 4.3, through the column removed a number (at least four) of different substances with varying absorption curves, presumably polynucleotides. These are being further investigated. Finally, treatment with 1 *N* HCl removed

[1] This investigation was supported in large part by a grant from the Rockefeller Foundation.

[2] Since submission of this paper, analogous procedures have been described by Hurst *et al.* (*J. Biol. Chem.*, **188**, 705 [1951]) and by Volkin *et al.* (*J. Am. Chem. Soc.*, **73**, 1533 [1951]).

[3] This resin was obtained through the courtesy of L. Matheson, of the Dow Chemical Company.

an additional 1% of the absorption placed on the column.

The yield of each fraction in terms of the ultraviolet absorption at 260 mμ initially placed on the column is summarized in Table 1. Of the initial absorption 95.7% was recovered.

TABLE 1

Fraction	Initial Absorption at 260 mμ (%)
Bases and nucleosides	21.6
Desoxycytidylic acids	10.8
Thymidylic acid	10.2
Desoxyadenylic acid	21.5
Desoxyguanylic acid	14.8
Eluted with 2 *M* buffer	15.8
Eluted with 1 *N* HCl	1.0
Total recovery	95.7

Ion exchange analyses of the digest resulting from the action of the pancreatic enzyme alone (for 80 hr at 37° C) have consistently revealed that only very small amounts of the four mononucleotides are released. Individually each accounts for less than 1% of the total initial ultraviolet absorption; over 95% of the absorption remains in the form of polynucleotides. This result is in agreement with the conclusions of Zamenhof and Chargaff (*6*).

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An Electronic Drinkometer

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The need for an apparatus for continuously recording the fluid intake of animals under a variety of experimental conditions has long been obvious in the study of thirst. Such a recorder is no less important, however, in other cases where fluid intake is measured, such as in studies of alcohol consumption or specific hungers for solutions of vitamins, salts, sugars, etc. The problem is to measure not only how much of a given fluid an animal drinks, but also when and how fast it drinks.

Various methods have been used in the past to record the course of drinking. The most obvious but most time-consuming is to take periodic readings of the fall of fluid in a graduated container. Another is to train the animal to operate a mechanism such as a lever-box in order to obtain measured amounts of fluid (*1*). A graph of the number of operations or number of lever pushes gives a complete record. But this method makes the animal work for its fluid and also rations the fluid in arbitrary amounts per response. A third method is the float-kymograph technique (*2*). The fall of fluid in a container is continuously recorded on a kymograph by a stylus that floats on the surface. The float-kymograph is a very cumbersome apparatus, and is also rather insensitive, since a sizable amount of fluid must be withdrawn from the system before the stylus is displaced.

The electronic drinkometer was designed to get around most of these shortcomings. The principle is simple. Whenever the animal touches the fluid it is to drink, it completes an electronic circuit that activates the pens of a kymograph. In the apparatus particularly designed for the rat, the main circuit consists of a 9-v battery source, a 20-megohm resistance, and a 6SN7 vacuum tube. One lead from this circuit goes to the wire-mesh floor of the rat's cage; the other makes contact with the fluid contained in an inverted graduate, fitted with a glass nipple from which the animal drinks. Every time the rat comes in contact with the fluid, it completes the circuit and biases the grid of the vacuum tube. Each change in grid bias fires a No. 850 advance relay, which closes another circuit to the magnets of a signal marker. Eight complete recording units of this sort were used to mark the waxed tape of a kymograph and thus provide permanent records of drinking.

In order to obtain the most sensitive records, the nipple of each graduate was brought up to a small opening in the wire-mesh wall of each cage so that only the rat's tongue could touch the fluid. As the rat drank, each tongue lap produced a single mark on the kymograph tape. With the tape running at 2.0 mm/sec, individual tongue laps could be counted, and the drinkometer could be calibrated for amount of fluid per tongue lap. At the slower tape speed of 0.5 mm/sec, used for prolonged recording, individual tongue laps could not be distinguished, and calibration was done in terms of the amount of fluid taken per millimeter of marked tape.

The actual process of calibrating was greatly facilitated by the fact that the rat's tongue always laps water at a constant rate of 6 or 7 laps/sec. This rate is maintained under a wide variety of conditions. For example, the rate is the same in the last 5 min of drinking in a 2-hr period as it is in the first 5 min. Furthermore, the rate is constant whether the rat has been deprived of water 6 hr or for as long as 7 days.

With the rate of lapping constant, it is possible to compute the total number of tongue laps made in a 2-hr period by measuring the amount of tape marked. The amount of water taken per tongue lap can then be calculated from the total water intake for that period, recorded to the nearest .5 ml from the graduated water bottles. The rats in this study averaged between .004 and .005 ml water/tongue lap. Essentially the same values were obtained when the rats were allowed to drink 1 or 2 ml water.

Variability in the amount of water taken per tongue lap was kept at a minimum in this work by holding the following factors as constant as possible: the size

of the rats, the size of the nipple openings, and the amount the animals had to stretch their tongues to reach the water. Male rats, about 5 months old, weighing around 350 g, were used. Nipple openings varied only between 3.6 and 3.9 mm, and each rat always drank from the same nipple. Finally, the nipples were always brought up to a fixed position at the cages so that tongue stretching was constant. As an added precaution to insure against wide rat-to-rat and condition-to-condition variations, the amount of water taken per tongue lap was always computed individually for every rat and every condition of drinking.

For practical purposes, measurement of drinking down to the last tongue lap is not necessary, of course. In fact, it is quite satisfactory in most cases to calibrate the drinkometer simply in terms of the amount of water ingested per millimeter of fully marked tape. With the kymograph going at 2.0 mm/sec, it can be shown that rats always drink at a rate of about 0.03 ml/sec. Even second-by-second records of drinking are too detailed for most purposes. It is often sufficient to determine the amount of drinking in each second and then make accurate minute-by-minute plots of the course of drinking (Fig. 1).

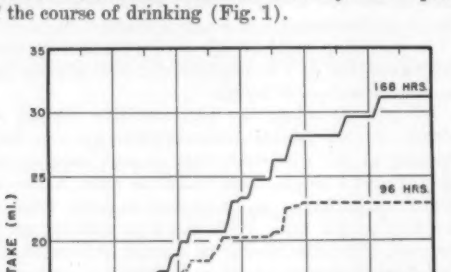

FIG. 1. Minute-by-minute plot of the rate of drinking of one rat following different amounts of water deprivation.

As Fig. 1 illustrates, the drinkometer yields detailed data which are very valuable in analyzing drinking behavior. It is clear that after water deprivation, the rat will drink virtually without interruption for as long as 8 min. Then it will rest and drink by turns. The more severe the deprivation, the less time the rat will spend resting and the longer it will drink each time it returns to the water bottle. Since drinking is always at a constant rate, the longer the water deprivation a rat has suffered, the more it will drink in a 2-hr period.

The drinkometer has also been used to trace the course of drinking over longer periods of time, up to 48 hr. In this case the kymograph tape was run at 0.5 mm/sec, and excellent records were obtained showing when the rat drinks and how much time it spends drinking during a normal day. The typical rat in our study does 78% of its drinking in the dark and devotes only about 20 min in every 24 hr satisfying its water needs.

The foregoing results illustrate only a few of the possible uses of the drinkometer. Nevertheless, they are sufficient to make several important features apparent. First, the drinkometer is simple, inexpensive, economical of space, and easy to operate. Second, it provides an extremely sensitive, continuous record of all the drinking an animal does over any reasonable period of time. Third, it will record fluid intake in any normal, free-drinking situation without disturbing the animal, making it work, or requiring that it be trained. Finally, although the drinkometer was designed for the rat, it could be used equally well with any mammal and probably any bird or reptile.

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The Role of Thiouracil in the Induction, Growth, and Transplantability of Mouse Thyroid Tumors

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Goitrogenic drugs, such as thiouracil, are believed to act directly on the thyroid gland to block reactions essential for the synthesis of thyroid hormone. The resulting diminished level of the thyroid hormone in the circulation is thought to provoke an increased output of thyrotrophic hormone (TSH) by the anterior lobe of the hypophysis, which acts on the secretory epithelium of the thyroid, producing cellular hypertrophy and hyperplasia. Thyroid glands of thiouracil-treated mice weighing 3–30 times more than those of control mice have been produced in this laboratory, but in spite of extreme hypertrophy involving both cellular hypertrophy and hyperplasia, no evidence of neoplasia was found in such thyroid glands (*1*). Nodules in the lungs, believed to represent true metastases from thyroid glands, were found, but such nodules did not display any more evidence of neoplasia than did the thyroid of the animal in which they occurred.

It was suggested by one of us (CDG) that prolonging the period of hyperplasia beyond the lifetime of any one mouse by transplanting the hyperplastic thyroid to other mice might result in further alteration, eventual neoplasia, and possibly even malignancy. The test was carried out by culturing thyroid tissue *in vivo* in a homologous growth medium where simultaneous

TABLE 1

ORIGIN AND TREATMENT OF THYROID TISSUES BEFORE AND AFTER TRANSPLANTATION

Expt. No.	Date original host started on thiouracil	Months on thiouracil before implantation	Date of original implantation	Original tissue implanted	Diet of host before implantation	Maximum No. implant generations*	Maximum time of thiouracil ingestion† (months)	Earliest appearance of complete autonomy‡ generation
177	9/14/44	16	11/30/45	Thyroid	145§	10	74	0
180	9/14/44	18	3/8/46	"	145	16	74	9
183	11/14/45	16	3/14/47	"	157‖	11	62	3
199	8/6/46	20	3/23/48	Lung metastatic nodule	160¶	4	51	0

* Transfer of original tissue to a new host represents beginning of the first implant generation. Numbers in this column refer to number of transplantations made subsequent to the original starting tissue.

† Includes time in original host plus time in transplant host.

‡ Complete growth autonomy means ability of tissues to continue growth without additional TSH stimulation, and began in the transplant generation indicated by the figures in this column.

§ Contained 0.5% thiouracil.

‖ 2-Acetylaminofluorene added at a level of 0.05% to the stock diet containing 0.5% thiouracil = diet 157.

¶ Diet 160 contained 0.375% thiouracil.

and continuous TSH stimulation was maintained indefinitely through ingestion of thiouracil. Small pieces of hyperplastic thyroid tissue from mice who had ingested thiouracil for many months (Table 1) were transplanted to young mice also ingesting thiouracil by means of sterile trocar. Every 3–6 months, or as soon as the implant had increased sufficiently in size, the tissue was retransplanted. The transplanted tissue developed an extensive vascular bed very rapidly and grew well. After a few transplantations, and when sufficient tissue became available, it was also implanted into mice receiving the stock diet devoid of the goitrogen. Thus it was possible to test frequently the growth potentialities or autonomy of the tissue in normal animals.

Three lines of thyroid tissue implants that have been produced by the above procedure are illustrated by experiments 177, 180, and 183 in Table 1. These implant lines originated from the thyroids of mice that had been fed thiouracil[1] for 16–18 months. Hyperplastic thyroid-cell growths and tumors, direct descendants of the original thyroids, have now been transferred through 10–16 generations over a period of 4–5 years (Table 1). Transplant line 183 is further complicated, however, because it originated from a mouse that also received .05% 2-acetylaminofluorene (AAF) in the thiouracil-containing diet. Although complete autonomy was first observed in this line with the third implant generation, and not until generation 9 in line 180, the possible relation of AAF thereto must await further study. A fourth thyroid tumor line, 199, originated from a thyroid metastasis transplanted from the lungs of a mouse after ingestion of thiouracil for 20 months (Table 1). This line has now been carried through 4 transplant generations, with growth potentialities similar to those from hyperplastic thyroids, even though it has not yet attained complete growth autonomy.

Several sublines have developed complete autonomy because increased TSH stimulation, induced by thiouracil ingestion, is no longer required for continued growth. Thyroid tumors often exceed 2 cm in diameter in 2–6 months, grow either intramuscularly or subcutaneously, and eventually kill the host. Metastases also occur in the lungs. One autonomous subline has been transferred to control mice for 10 consecutive generations. The histologic structure of the transplants of this tumor subline carried in hosts receiving thiouracil was in general similar to that of the transplants carried in hosts fed the control diet (*2*). Other sublines still require TSH stimulation, and when such stimulation is withdrawn, by removing thiouracil from the diet of the host, the implant undergoes regression.

Recently Hall and Bielschowsky (*3*) reported morphological evidence that malignancy also appeared in the thyroid of rats receiving a goitrogen for 1½ years. These investigators concluded that the essential factor in the pathogenesis of the experimental tumors of the rat thyroid was the continuous increased stimulation by TSH. Money and Rawson (*4, 5*) consider that, in addition to the over-stimulation of the thyroid by TSH, the iodine deficiency produced through the action of antithyroid drugs may also be involved in the process of thyroid tumor formation in rats.

Bielschowsky *et al.* (*6*) have transplanted the experimentally produced thyroid tumors of rats only when a state of thyroid deficiency was maintained with its consequent increased output of TSH, which shows that the tumors were not autonomous, but could be maintained only under the same conditions responsible for the production of the original tumor.

The work reported here is believed to furnish the first experimental evidence that autonomous thyroid tumors can be produced in mice after prolonged ingestion of thiouracil and presumably similarly pro-

[1] The thiouracil used in these experiments was generously supplied by Lederle Laboratories, Pearl River, N. Y., through the courtesy of S. M. Harding.

longed TSH stimulation. The fact that the mouse thyroid tissues were continued for a longer period in a continuous state of stimulation than were those of the rat may be one explanation for the successful development of thyroid tumors in mice transplantable to normal animals. Wollman, Morris, and Green (*7*), in preliminary studies of the functional activity of 4 tumor sublines, found that 4 transplantable tumors showed a wide variation in ability to concentrate inorganic iodide or to convert it into thyroxine. These studies are suggestive of the potential value of experimental thyroid tumors as an aid to a better understanding of thyroid physiology and cancer. The peculiarities of several sublines of thyroid implants and autonomous tumors in relation to their functional, histological, and cytological characteristics will be reported in detail elsewhere.

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Comments and Communications

Radioactivity of the Hot Springs of Tiberias[1]

Several experiments that have been carried out in the past (M. Buchmann, *J. Palestine Med. Assoc.*, **25**, [8], 4 [1943]) appeared to indicate that the thermal springs located at Tiberias, Israel, are radioactive. In view of the fact that remarkable curative properties have been ascribed to these springs since antiquity, a new investigation, using the improved techniques that have become available during the past decade, has been initiated.

The springs issue at a temperature of 63° C. Their chief constituents, by weight, are the dissolved chlorides of sodium, calcium, and magnesium. Kodak N.T.A. plates with an emulsion thickness of 25 μ have been exposed by immersion in samples of water from the springs, in samples that were evaporated down to various increased concentrations, and in solutions of salts crystallized from the springs. Exposures were also effected by several other methods, including suspension of plates above the springs and evaporating samples on the surface of the plates. The age of samples varied from a few hours to several years.

Some of the exposures resulted in the appearance of peculiar structures. The dimensions and number per unit area of the phenomena were such that they could reasonably be attributed to α-radiation, but their spurious nature became evident upon close examination. Consequently, in the case of the relatively few exposures in which these pseudo tracks and stars appeared, they were excluded from the α-count.

Counts of α-tracks and stars failed to indicate any consistent activity above background. Control was maintained by addition of minute amounts of very weak standard solutions of radium barium bromide to samples and noting the increased α-counts. These control experiments indicate that samples emitting α-radiation energetic enough to produce discernible tracks—i.e., $E > 1.7$ mev, and of an activity exceeding 10^{-13} curies/cm^3—would have yielded statistically significant net counts. β-radiation, as well as short half-life, or very low-energy α-activity would have escaped detection in these experiments.

Further research, making use of several types of counters, is being carried out in an effort to check on these possibilities, as well as to revise the present upper limit to the α-activity.

[1] Research supported by the F. Julius Fohs Foundation.

David B. Rosenblatt

Hebrew Institute of Technology
Haifa, Israel

Climate and Culture—New Evidence

In 1932 (*1*) I suggested that the remarkable flowering of the mound-building cultures in the upper Mississippi and Ohio drainage had been coincident with a prolonged warm-dry phase of climate to which the name xerothermic has been applied. This period had been marked by a shift of continental vegetation, including grasslands, toward the Northeast. Such a movement was deduced by Gleason (*2*) on the basis of plant distribution, and later amply confirmed by pollen analysis of fresh-water sediments.

I also suggested that the replacement of the mound cultures by less advanced forest cultures was related to the subsequent cooling and increase of humidity. These conditions favored the return of forests.

Since 1948, with the active encouragement of Mexican colleagues and financial assistance from the Geological Society of America and the Wenner-Gren Foundation (Viking Fund), I have had an opportunity to study pollen sequences in relation to archaeology within the Mexico City Basin. This study has established the fact that the transition Late Archaic–Teotihuacan I took place during a prolonged dry period whose position in the sequence corresponds to our xerothermic.

The recent carbon 14 dating by Arnold and Libby (*3*) assigns this transition and the Hopewell mound

culture to about the same period—*ca.* 400–500 B.C.—and the Adena mound material to a date—*ca.* A.D. 800—not far from the end of Teotihuacan as usually accepted. Thus the Hopewell-Adena span appears to correspond roughly to the Teotihuacan period in Mexico—an interval during the early part of which the basin floor below Teotihuacan seems not to have been the scene of much cultural activity.

It is true that some Archaic mound material (antler and shell) from Kentucky has been assigned an antiquity of the order of 3000 B.C. This naturally raises the question of its relation, ethnic and cultural, to the much later Hopewell. On internal evidence the Mexican affinity of the latter seems strong. Was this affinity an effect of mass migration, or of a more subtle influence?

At any rate, it is now clear that the great flowering of the Ohio Mound cultures took place during the xerothermic, when dry climate, minimum lake levels, and suspended activity characterized the inner Basin of Mexico City.

This situation offers a remarkable parallel to the intrusion of cereal culture in Scandinavia during the warm dry sub-boreal (*4*). So far as I can tell, the latter corresponds in position to our xerothermic, but how closely it corresponds chronologically can only be determined by further research.

PAUL B. SEARS

Yale University

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Illiteracy Triumphant

CHESTER LONGWELL certainly deserves hearty congratulations for his letter, "The 1950 Silly Season," published in the April 13 issue of SCIENCE. Many readers must have been as delighted as I was with his pungent remarks about the *Saturday Evening Post's* inspired editorial in defense of Galileo, Velikovsky, and *Worlds in Collision*.

Now for the next important point—where do we go from here? A good chuckle and a well-directed barb may be enough for Dr. Longwell, but not for me. The editorial was silly. As a work of scientific research, *Worlds in Collision* ranks with Grimm's fairy tales and the *Rubaiyat*. The increasing popularity of pseudo science is a matter for the deepest concern.

These truths are self-evident in the columns of SCIENCE. But isn't the problem important enough for more than a few semiprivate communications among friends, or an occasional biting book review? Has the shift of publishers from Macmillan to Doubleday (which has no soft underbelly in the form of a textbook department) improved the situation in any fundamental sense? Can scientists do more than they have done, and take positive steps through their professional societies?

The above questions are only partly rhetorical. I feel that something more ought to be done; perhaps others will not consider it worth the trouble. But I am reminded of the biologists and physicians who are taking aggressive action against the antivivisectionists. Also, the American Psychological Association has spoken out mildly against dianetics and "engrams."

Why haven't the astronomers, linguists, geologists, or anthropologists—speaking through their societies—come out with their feeling about *Worlds in Collision*? Or should that be the function of the AAAS? If not, is there an organization that represents the body of American science in such matters?

The pseudo-science enthusiasts have excellent public relations. Science has no public relations to counteract them. We are justly proud of the fact that the standards of popular science reporting as represented in the National Association of Science Writers are as high as any in the world. But it seems to me that scientists and their official representatives must take the initiative in combating pseudo science—and, on the positive side, presenting science and the scientific method in the best possible light.

This would not be an easy task, even if we were not on the verge of a dangerous reaction against research and rational thinking of all kinds. The difference between Galileo and Velikovsky hardly needs arguing here. But it may be helpful to shift our perspective for a moment. An American who has not been trained in science may sincerely feel that Galileo and Velikovsky are both martyrs of "authoritarian" tendencies. Imagine that we have to show him the error of his ways—and, sooner or later, we may have to do just that. What shall we tell him, and how shall we put it? We must be as articulate and brief as that *Saturday Evening Post* editorial which, for all its silliness, was clearly written in straightforward language.

Science has a message beyond the results it has achieved in medicine and technology. It has not yet succeeded in communicating this message to the American public, and, until it does, pseudo science will continue to find a wide audience. Perhaps some publishers, authors, and scientifically illiterate critics deserve the lion's share of the blame. But scientific societies deserve a brickbat or two. They know the facts—and have maintained a dignified silence.

JOHN PFEIFFER

34 W. 73rd St., New York

(Editorial Note: *The editors disclaim any dignity in the silence maintained in* SCIENCE *until Chester E. Longwell's comment was published. They have been only too mindful of the box-office successes and best-seller records of plays and books that have been literary and financial failures until some society, watchful of public morals, gave them the boost that paid off. There is, however, cause for alarm that there are publishers and editors so scientifically illiterate as to appear unable to differentiate between fact and fiction—authentic science and hoax.*)

On Requesting Chemicals

INDUSTRIAL laboratories frequently receive requests from investigators for chemicals that may be available. These requests are welcome, but observance of the three simple rules set forth below would speed the filling of such requests and save considerable correspondence.

1) State the amount of material needed in the first inquiry. "Small" is a relative term.

2) Ask for no more than the amount actually required. Unused chemicals on many shelves dissipate rare material.

3) Check the catalogues of suppliers of organic chemicals before making the request.

JACKSON P. ENGLISH

Chemotherapy Division
Stamford Research Laboratories
American Cyanamid Company
Stamford, Connecticut

Zoological Nomenclature: Notice of Proposed Suspension of Rules in Certain Cases for Avoidance of Confusion and Validation of Current Nomenclatorial Practice (A. (n.s.) 8)

NOTICE is hereby given that the possible use by the International Commission on Zoological Nomenclature of its plenary powers is involved in applications relating to the undermentioned names included in Parts 5 to 8 of Volume 2 of the *Bulletin of Zoological Nomenclature,* each of which parts was published on May 4, 1951:

(1) *Applications in Part 5*

1) *Titania* Meigen, 1800, and *Chlorops* Meigen, 1803 (Class Insecta, Order Diptera), and associated family names (pp. 134–38) (File 197).
2) *Dorilas* Meigen, 1800, and *Pipunculus* Latreille [1802–1803] (Class Insecta, Order Diptera), and associated family names (p. 140) (File 221).
3) *Tendipes* Meigen, 1800, and *Chironomus* Meigen, 1803 (Class Insecta, Order Diptera), and associated family names (pp. 150–51) (File 469).
4) *Philia* Meigen, 1800, and *Dilophus* Meigen, 1803 (Class Insecta, Order Diptera), (pp. 153–54) (File 498).
5) *Tylos* Meigen, 1800, and *Micropeza* Meigen, 1803 (Class Insecta, Order Diptera), and associated family names (pp. 156–59) (File 501).

(2) *Applications in Triple-Part 6/8*

6) *Sphaeroceras* Bayle, 1878 (Class Cephalopoda, Order Ammonoidea), proposed validation of, by suppression of *Sphaeroceras* Hope, 1840 (Class Insecta, Order Coleoptera), (pp. 164–66) (File 405).
7) *Procerites* Siemiradzki, 1898 (Class Cephalopoda, Order Ammonoidea), proposed designation of type species of (pp. 167–69) (File 403).
8) *Macrocephalites* Zittel, 1884, proposed designation of type species of; *Ammonites macrocephalus* Schlotheim, 1813, proposed determination of species to which name applicable (Class Cephalopoda, Order Ammonoidea) (pp. 170–72) (File 401).
9) *Pictonia* Bayle, 1878, and *Rasenia* Salfeld, 1913, proposed designation of type species of; trivial name *cymodoce* d'Orbigny, 1850 (as published in the binominal combination *Ammonites cymodoce*), (Class Cephalopoda, Order Ammonoidea), proposed suppression of (pp. 178–80) (File 421).
10) *Aulacostephanus* Tornquist, 1896 (Class Cephalopoda, Order Ammonoidea), proposed designation of type species of (pp. 188–90) (File 384).
11) *Kosmoceras* Waagen, 1869, *Harpoceras* Waagen, 1869, and *Perisphinctes* Waagen, 1869 (Class Cephalopoda, Order Ammonoidea), proposed designation of type species of (pp. 191–93) (File 445).
12) *Planites* de Haan, 1825, proposed suppression of; *Nautilus polygyratus* Reinecke, 1818 (Class Cephalopoda, Order Ammonoidea), determination of species to which name applicable (pp. 194–97) (File 402).
13) *Planulites* Lamarck, 1801, *Orbulites* Lamarck, 1801, *Pelagus* Montfort, 1808, *Ellipsolithes* Montfort, 1808, *Globites* de Haan, 1825 (Class Cephalopoda, Order Ammonoidea), proposed suppression of (pp. 198–99) (File 423).
14) *Ammonites* Bruguiere, 1789 (Class Cephalopoda, Order Ammonoidea), proposed suppression of (pp. 200–203) (File 425).
15) *angulatus* Schlotheim, 1820 (as published in the binominal combination *Ammonites angulatus*) (Class Cephalopoda, Order Ammonoidea), proposed validation of, and determination of species to which name applicable (pp. 204–207) (File 422).
16) *Tozosphinctes* Buckman, 1923 (Class Cephalopoda, Order Ammonoidea), proposed suppression of (in favor of *Arisphinctes* Buckman, 1924) (pp. 214–16) (File 389).
17) *Arnioceras* Hyatt, 1867 (Class Cephalopoda, Order Ammonoidea), proposed designation of type species of (pp. 217–19) (File 509).
18) *Liparoceras* Hyatt, 1867 (Class Cephalopoda, Order Ammonoidea), proposed designation of type species of (pp. 220–22) (File 507).
19) *Normannites* Munier-Chalmas, 1892 (Class Cephalopoda, Order Ammonoidea), proposed designation of type species of (pp. 222–23) (File 508).
20) *virgula* Deshayes, 1831 (as published in the binominal combination *Gryphaea virgula*) (Class Pelecypoda), proposed validation of (pp. 234–35) (File 407).
21) *asper* Lamarck, 1819 (as published in the binominal combination *Pecten asper*) (Class Pelecypoda), proposed validation of (pp. 236–37) (File 408).
22) *Gryphaea* Lamarck, 1801 (Class Pelecypoda), proposed suppression of, and validation of *Gryphaea* Lamarck, 1819 (pp. 239–40) (File 365).

2. The present notice is given in pursuance of decisions taken, on the recommendation of the International Commission on Zoological Nomenclature, by the Thirteenth International Congress of Zoology, Paris, July 1948 (*Bull. Zool. Nomenclature,* 4, 51, 57 [1950]; *ibid.,* 5, 5, 131).

3. Any specialist who may desire to comment on any of the foregoing applications is invited to do so in writing to the Secretary to the International Commission, 28 Park Village East, Regent's Park, London,

N. W. 1, Eng., as soon as possible. Every such comment should be clearly marked with the commission's file number as given in the present notice.

4. If received in sufficient time before the commencement by the International Commission of voting on the applications in question, comments received in response to the present notice will be published in the *Bulletin of Zoological Nomenclature*; applications received too late to be so published will be brought to the attention of the International Commission at the time of the commencement of voting on the application in question.

5. Under the decision by the International Congress of Zoology specified in paragraph 2 above, the period within which comments on the applications covered by the present notice are receivable is a period of 6 calendar months calculated from the date of publication of the relevant part of the *Bulletin of Zoological Nomenclature*. The four parts now in question were published on May 4, 1951. In consequence, any comments on the applications published in those parts should reach the Secretariat of the International Commission at latest by *November 4, 1951*.

FRANCIS HEMMING

Secretary to the International Commission on Zoological Nomenclature

Erratum

WE HAVE been informed that reference 14 in the article by Eugene L. Hess (*Science*, 113, 709 [1951]) is to the new journal *Arkiv för Kemi* and not to *Arkiv Kemi, Mineral., Geol.*—EDITORS.

Book Reviews

The Wealth of India: A Dictionary of Indian Raw Materials and Industrial Products; Raw Materials, Vol. II, and ***Industrial Products,*** Part II, covering letter "C." B. N. Sastri, Ed. New Delhi, India: Council of Scientific & Industrial Research, 1950, 1951. Vol. II, 427 pp., Rs. 25, or about $5.25; Part II, 251 pp., Rs. 15, or about $3.15.

This is the second volume in the continuing series dealing with the raw materials and industrial products of India. It consists of two books, in which are described all subjects starting with the letter "C." Volume I, published in December 1948, covers subjects starting with the letters "A" and "B." It is understood that the series will cover over 4,000 subjects, about 90 per cent of which will relate to plant products, and the remainder to mineral products, mineral resources, and industries.

The work is an encyclopedia with subjects arranged in alphabetical order, and appears to be the result of a painstaking review of the literature and compilation of statistics. The information is presented in a manner understandable and useful to both layman and scientist. The text is well illustrated with line drawings, graphs, charts, colored plates, and half tones. The printing on heavy glossy paper is large and clear. However, because of the weight of the book it is doubtful if the binding will stand much hard usage. A vast variety of subjects, such as cotton, copper, cosmetics, chrysanthemum, cutlery, coal carbonization, ceramics, canvas, crocus, etc., is covered, and general, specific, and scientific information is given on each. The description of "Chromite," for example, occupies 9 pages and includes sections entitled "Occurrence," "Distribution," "Mining and Dressing," "Grading and Marketing," "Industrial Uses," "Scope for Expansion;" that of "Camellia" (from the leaves of which tea is made) covers 24 pages and includes a description of various species and sections on "Cultivation," "Manufacture," "Composition," "Production and Trade." The description of each subject generally begins with its scientific name, including related species, followed by its physical and chemical characteristics. Brief mention is made of the most important world occurrences and then a detailed description of where and how the subject is found in India and how it is used industrially. Numerous references are given throughout the text.

Undoubtedly one of the prime purposes of the Indian government in going to the great cost and effort of compiling this encyclopedia is to provide accurate and concise information about the natural resources of the country in order to encourage their development. The Council of Scientific and Industrial Research maintains an Informational Bureau of Natural Resources to answer inquiries from India and abroad regarding the scientific aspects, production, uses, sources of supply, and trade statistics of Indian raw materials and industry.

The books are excellent for reference, general reading, and teaching and should be in all libraries, universities, and other scientific institutions. They would be valuable also for commercial firms doing business in India.

A. F. BANFIELD

New York City

Oral Pathology. 3rd ed. Kurt H. Thoma. St. Louis: Mosby, 1950. 1,592 pp. $17.50.

In this revised and enlarged third edition of his well-known textbook, the author presents the pathogenesis, diagnosis, histopathology, and treatment of anomalies, diseases, and tumors of the mouth and jaws. The volume covers in most instances the clinical symptoms, x-ray studies, treatment, and, where indi-

cated, the histopathology of the various lesions. The 1,660 carefully selected illustrations, of which 78 are in color, are well labeled and described.

Dr. Thoma is unusually considerate in his use of synonyms, so that all readers may follow without continual reference to other sources. He presents not only his theories and views upon a particular subject but nearly always the work and thoughts of at least one other investigator. The book also contains a splendid chapter on periodontal disease. A simple, understandable classification based on clinical findings is well presented and described.

This text embraces recent investigation and research in oral pathology. It contains revised classifications of several disease processes and justifies their use. Of particular interest is the chapter on odontogenic tumors, which is essentially the same as that approved by members of the American Academy of Oral Pathology at their meeting in May 1950 at the Armed Forces Institute of Pathology.

A wealth of reference material is included in each chapter. Not only does it give Dr. Thoma authority for his work but it is also invaluable for those who wish to read further.

The author correlates this book with his two volumes on oral surgery, and he treats developmental pathology much more thoroughly than most authors. He also has an excellent chapter on abnormal occlusal relationship of the dentures. For this reason, and the fact that these entities are mentioned and discussed throughout the book, and in relation to other pathology, this book must be of particular value to the orthodontist as well as to the clinician, the oral pathologist, and the surgeon.

JOSEPH L. BERNIER, Col. (DC), USA
Dental and Oral Pathology Section
Armed Forces Institute of Pathology

Studies in Late Tertiary Paleobotany. Daniel I. Axelrod. Washington, D. C.: Carnegie Institution of Washington, 1950. 323 pp., 19 plates. $2.75 paper; $3.25 cloth.

This volume represents another milestone in Dr. Axelrod's studies of West Coast floras begun in the thirties. The first of the 6 papers, "Classification of the Madro-Tertiary Flora," presents a revision of these floral units. The author proposes to divide the major Tertiary floras (Madro-Tertiary, Arcto-Tertiary, etc.) into complexes, which, in turn, are divided into elements and components (two terms proposed by Chaney in 1944). Dr. Axelrod demonstrates the necessity for recognizing these units in studies of fossil floras.

The next 4 papers describe and interpret Pliocene floras from California. Three floras are described for the first time: the Napa from central California, and the Anaverde and Piru Gorge from southern California. The fourth paper presents the results of a restudy of the southern California Mount Eden flora first investigated by the author in 1934 and 1937. Six new species are described. All these papers illustrate the unique methods and principles of paleobotany, many of which were formulated earlier by Dr. Axelrod (1941, 1949).

The final study, "Evolution of Desert Vegetation," presents a different view of this problem. The author's thesis is that living desert species are derived from more mesic Tertiary communities which occupied the areas of present-day desert, and that the living species developed from these Tertiary communities by adapting themselves to a climate which was becoming progressively drier throughout Tertiary time. From paleobotanical evidence, the author argues convincingly that modern deserts are a product of Upper Pliocene and Pleistocene time. From paleobotanical evidence, distributional patterns, and modern floral associations, he points out that the remarkable similarities between the North and South American living floras apply to less than 1 per cent of the living flora of each of these continents, and that this phenomenon can be explained by differentiation from wider-ranging ancestors, and by long-distance migration at times of favorable environmental conditions.

This volume is eloquent proof of the statement that the fossil floras of the western United States are the best known in the world.

ARTHUR A. MEYERHOFF
Palo Alto, California

Scientific Book Register

Physical Geography. Arthur N. Strahler. New York: Wiley; London: Chapman & Hall, 1951. 442 pp. $6.00.

An International Bibliography on Atomic Energy: Scientific Aspects, Vol. 2. New York: Atomic Energy Commission Group, United Nations, 1951. Distributed by Columbia Univ. Press, New York. 24,282 listings with introductory essays and appendices. $10.00.

Aircraft Jet Powerplants. Franklin P. Durham. New York: Prentice-Hall, 1951. 326 pp. $5.00.

The Integument of Arthropods: The Chemical Components and Their Properties, the Anatomy and Development, and the Permeability. A. Glenn Richards. Minneapolis: Univ. Minnesota Press, 1951. 411 pp. $6.00.

A Treatise on Heat. Including Kinetic Theory of Gases, Thermodynamics and Recent Advances in Statistical Thermodynamics. 3rd ed. M. N. Saha and B. N. Srivastava. Allahabad and Calcutta, India: Indian Press, 1950. 935 pp. Rs. 32/–.

Technique of Organic Chemistry: Adsorption and Chromatography, Vol. V. Harold Gomes Cassidy; Arnold Weissberger, Ed. New York-London: Interscience, 1951. 360 pp. $7.00.

The Kidney: Structure and Function in Health and Disease. Homer W. Smith. New York: Oxford Univ. Press, 1951. 1,049 pp. $12.50.

The Kidney: Medical and Surgical Diseases. Arthur C. Allen. New York: Grune & Stratton, 1951. 583 pp. $15.00.

Johannes Kepler: Life and Letters. Carola Baumgardt. New York: Philosophical Library, 1951. 209 pp. $3.75.

Servomechanisms and Regulating System Design, Vol. I. Harold Chestnut and Robert W. Mayer. New York: Wiley; London: Chapman & Hall, 1951. 505 pp. $7.75.

News and Notes

Scientists in the News

Cole Coolidge has been appointed director of the Chemical Department of the Du Pont Company, succeeding **Elmer K. Bolton,** who is retiring after 21 years as director. Dr. Coolidge has been assistant director of the department since 1939.

George Cooper, Jr., associate professor of roentgenology, University of Virginia, has been awarded the American Cancer Society's 1951 Medal in recognition of his contributions to the control of cancer. Dr. Cooper is medical and scientific director and a member of the Board of Trustees of the Virginia Division, American Cancer Society, and chairman of the cancer committee of the Medical Society of Virginia.

Walter G. Daniel, professor of education at Howard University, has been made specialist for higher education in the Office of Education, FSA. He will work with **John Dale Russell,** assistant commissioner for higher education, on improvement of the nation's program of higher education.

Fratis L. Duff has been appointed chief of the Preventive Medicine Division, Office of the Surgeon General, USAF. Reassigned from a teaching post at Johns Hopkins School of Medicine, he has devoted a major part of his career to the field of preventive medicine.

Paul X. English, U. S. A. (ret.), has been appointed director of industrial engineering for Guy B. Panero, Engineers. General English was formerly wartime chief of the industrial division of the Chemical Corps. Before joining Panero, he was with the Harmonic Reed Corporation, Philadelphia.

M. P. Etheredge has suceeded **Clay Lyle** as dean of the School of Science at Mississippi State College. Dr. Etheredge has been state chemist, professor of chemistry, and head of the Department of Chemistry at Mississippi State. In addition, he is serving as president of the Association of American Feed Control Officials. Dr. Etheredge was referee on starch for the Association of Official Agricultural Chemists, 1940–44, and on nitrogen in fertilizers, 1947–50. He is now serving as general referee on feeding stuffs. He is past president of the Food and Drug Officials of the South Central States, and he represents Mississippi State College on the council of the Oak Ridge Institute of Nuclear Studies.

James L. Fieser, former executive director of the Committee for a Christian University in Japan, has been appointed chief of the Mass Care Planning Branch, Health and Welfare Services Office, of the Federal Civil Defense Administration. Since 1947, Mr. Fieser has been director of the Suburban Hospital in Bethesda, Md.

Chester Hume Forsyth, a member of the Mathematics Department of Dartmouth College for 35 years, retired at the end of the college year. Before his appointment as an instructor in mathematics at Dartmouth in 1916, Professor Forsyth had taught at the University of Illinois, the University of Michigan, and Eureka College.

The Max Planck medal, highest award of the German Physical society, has been given to **James Franck,** professor emeritus of physical chemistry in the University of Chicago's Institute for Radiobiology and Biophysics. Although he began as a physicist, Professor Franck in recent years has devoted himself to a study of the effects of photosynthesis. His studies at the University of Chicago have been supported by the Samuel S. Fels Fund. The Max Planck medal has been awarded annually since the middle 1920s.

S. I. Griboff, of New York, serving his internship at New York City's Mount Sinai Hospital, has won the Schering Award for 1950. Dr. Griboff was a senior medical student at Syracuse University College of Medicine when he enrolled in the competition, and received first prize for his paper on "The Clinical Use of Steroid Hormones in Cancer." Co-authors **Robert W. Winters** and **Henry M. Williams,** students at Yale University School of Medicine, were selected as a tie for second prize with **Kenneth J. Ryan,** student at Harvard Medical School; **Monte J. Meldman,** student at Marquette University School of Medicine, was awarded third prize. Twenty-five contestants received honorable mention. The Schering Award has been given annually for the best manuscripts prepared on designated phases of endocrinology. Medical students of the United States and Canada are eligible for the contest. Judges for 1950 were Ira T. Nathanson, assistant professor of surgery, Harvard Medical School; Cornelius P. Rhoads, director of the Memorial Center for Cancer and Allied Diseases, New York; and Walton W. Van Winkle, former secretary of the Committee on Research of the Council on Pharmacy and Chemistry of the AMA.

M. I. Grossman, associate professor of clinical science at the University of Illinois College of Medicine, on military leave of absence, has reported for service at Fort Sam Houston. He holds the rank of captain in the Army Medical Corps. Dr. Grossman is also assistant managing editor of *Gastroenterology,* and research associate at the Hektoen Institute for Medical Research.

A Swedish expedition to New Guinea will be led by **Nils Gyldenstolpe,** of the Swedish Museum of Natural History. He will explore the little-known central plateau of the island, hoping to find rare mammals and birds.

Halsted S. Hedges, emeritus professor of ophthalmology at the University of Virginia, was honored for

his 52 years of service by the Virginia Society of Ophthalmology and Otolaryngology at its annual meeting in Roanoke. Dr. Hedges, the first member of the society to be so honored, has been a member of the medical faculty of the University of Virginia since 1899.

George E. Holbrook, manager of the plant development section of Du Pont's Organic Chemicals Department, has been named assistant director of the company's Development Department. Dr. Holbrook has been with Du Pont since 1933, starting as a research chemist in the Jackson Laboratory, Deepwater Point, N. J.

Edwin P. Jordan, former director of education of the Frank E. Bunts Educational Institute and the Cleveland Clinic Foundation, has been appointed executive director of the American Association of Medical Clinics.

The first $10,000 C. C. Criss Award and gold medal for "outstanding contributions in the fields of public health and safety" were awarded to **Edward C. Kendall** and **Philip S. Hench,** last year's Mayo Clinic Nobel prize winners. The award was established in 1949 by Mutual of Omaha, health and accident insurance company, and is not limited to medical men. Dr. Kendall will join the Forrestal Research Center at Princeton this year as visiting professor of chemistry.

Margaret A. Kennard has left the University of Oregon Medical School to accept an appointment as associate professor of physiology at the University of British Columbia. She will also serve as senior researcher at Crease Psychiatric Clinic Research Unit.

Ching Chun Li, a specialist in population genetics, has been appointed a research fellow in biostatistics at the University of Pittsburgh Graduate School of Public Health. Under the Communist regime, Dr. Li was obliged to leave his position as professor and head of the Department of Agronomy at National Peking University, Peiping. He was enabled to return to the U. S. through the efforts of the Committee on Aid to Geneticists Abroad of the Genetics Society of America.

C. N. Hugh Long, dean of the Yale School of Medicine, received the 1951 Banting Memorial Medal from the American Diabetes Association at its annual meeting. As medal recipient, Dr. Long delivered the Banting Memorial Lecture, on "The Endocrine Control of the Blood Sugar." The medal and lectureship were established in 1941 in memory of Frederick G. Banting, the Canadian scientist who was the co-discoverer of insulin.

Perrin H. Long has been made professor of medicine and executive officer of the Department of Medicine of the College of Medicine at New York City, State University of New York.

C. E. Marshall, professor of soils, College of Agriculture, University of Missouri, was recipient of the first Hoblitzelle National Award in Agricultural Sciences ($5,000 cash and gold medal), created to give recognition to basic research in the agricultural sciences by Karl Hoblitzelle, Texas philanthropist. Dr. Marshall was chosen from a list of more than 70 scientists nominated from all parts of the U. S. The Final Awards Committee was composed of T. S. Painter, president of the University of Texas; William A. Albrecht, University of Missouri; P. V. Cardon, Agricultural Research Administration, USDA; Louis Bromfield, writer and farmer; and J. E. Coit, consulting horticulturist from California.

Marston Morse, of the Institute for Advanced Study, has been made chairman of the new NRC Division of Mathematics. **Marshall Stone,** of the University of Chicago, will serve as vice chairman, and **J. R. Kline,** of the University of Pennsylvania, as executive secretary.

Richard P. Saunders has joined the Save the Children Federation as president-elect. He will become president on January 1, succeeding **John R. Voris,** who will retire after 20 years of service. Dr. Saunders has been president of Palos Verdes College, Rolling Hills, Calif., since 1946.

Gordon Merritt Shrum, head of the Department of Physics, University of British Columbia, and **Andrew Robertson Gordon,** head of the Department of Chemistry, University of Toronto, have been appointed members of the Defence Research Board of Canada.

Harold M. Skeels, senior scientist, USPHS, has been assigned to the National Institute of Mental Health, Bethesda, Md., as special projects services consultant in the Community Services Branch. For the past two years Dr. Skeels has served as PHS regional mental health consultant in clinical psychology, San Francisco.

L. E. Sutton, of the Physical Chemistry Department, Oxford University, has been invited to give a course of lectures at the Harvard University Summer School. He will attend the fall jubilee meeting of the American Chemical Society before he returns to England.

Hugh S. Taylor, dean of the Princeton University Graduate School and former chairman of the university's Department of Chemistry, was selected to present the 1951 Remsen Memorial Lecture of the American Chemical Society's Maryland Section. Dean Taylor, an authority on catalysts, photochemistry, and the nature of chemical processes at the surfaces of solids, is the sixth American chemist to receive this honor since the lecture series was established in 1946, in memory of Ira Remsen, first professor of chemistry at Johns Hopkins.

William F. Taylor has succeeded **Max Halperin** as head of the Department of Biometrics at the Air Force School of Aviation Medicine, Randolph AFB. The latter has been transferred to Washington, D. C., as statistician for the U. S. Public Health Service.

Education

The **University of Florida** will offer for the first time work leading to the Ph.D. degree in plant pathology and mycology and applied phases, beginning with the fall semester. The teaching and research staff will include 25 pathologists of wide experience. Special emphasis is to be placed on subtropical and tropical fungi and plant diseases, as well as diseases of so-called Southern crop plants, including mostly diseases of ornamental plants, citrus, vegetable, and field crops. Graduate assistant and teaching assistant fellowships are available.

Illinois Institute of Technology has been awarded a contract for research on multi-story apartment building construction by the Housing and Home Finance Agency. Under the direction of Elmer I. Fiesenheiser, and supervised by the agency's Division of Housing Research, work will be done by the Civil Engineering Department on the development of low-cost methods of construction of low-rent housing through economies in materials, use of space, structural and mechanical design, assembly and installation of equipment. The Structural Clay Products Research Foundation is also planning to assist in the project.

The **University of Kansas** Medical School, in reorganizing its Department of Pathology, will combine it with the Department of Oncology. Robert E. Stowell, head of the latter department, will succeed H. R. Wahl, who will retire as chairman of the Department of Pathology. New medical school appointments include Harlan I. Firminger, Chauncey G. Bly, David M. Gibson, Betty U. Corbett, and Max Berenbom. A Medical Science Building is under construction to provide additional space in 1952.

Northwestern University Medical School will combine its Departments of Chemistry and Experimental Medicine into a Department of Biochemistry. Smith Freeman, who has been professor and chairman of the Department of Experimental Medicine, will head the new department.

In conjunction with the alumni reunion program at the **University of Notre Dame,** the cornerstone was laid for a new Science Building, which is scheduled for completion next summer. It will house the departments of Chemistry, Physics, and Mathematics.

Ohio State University is initiating a curriculum in sanitary engineering leading to a B.S. in civil engineering. Among the specialized courses, to begin in the third year, are sanitary bacteriology, environmental sanitation, advanced hydraulics, water- and sewage-treatment plant design, and treatment of industrial wastes. Between the third and fourth years, three weeks will be spent at the summer surveying camp in Zaleski State Forest and six with the Ohio Department of Health in a field training program.

Peabody Museum has sent four anthropologists to Point Barrow, Alaska, to search for archaeological remains of aboriginal Americans and to study the present-day culture of the Alaskan Eskimos. Wilbert K. Carter, of Big Timber, Mont., is head of the expedition, which is sponsored by ONR. He will be assisted by Charles I. Shade, H. Thomas Cain, and Robert W. Tanner.

The newly organized **University of Ryukyus,** near Naha, Okinawa, will add five Michigan State professors to its staff for the academic year 1951–52. Last year, the first year of the university's operation, more than 500 students were in attendance. Major emphasis is on the practical arts, mainly agriculture and home economics, on the teaching of English, and on informational and educational programs among adults. The American Council on Education supervises the entire aid program.

Thirty **Tulane University** students and six professors, interested mainly in advanced work in anthropology, are attending a special summer school in Guatemala, with the cooperation of the University of San Carlos and the U. S. Embassy.

Vermont Agricultural College has received by transfer from the U. S. government the 942-acre U. S. Morgan Horse Farm in Addison County, near Middlebury. The property is to be used for research on "animals and animal products, plants, soils, and forestry; farm demonstrations; and any phase of agricultural education."

Wesleyan University has joined twelve other liberal arts colleges (*Science,* **113,** 706 [1951]) in the Columbia University School of Engineering program designed to provide engineering students with a combined liberal arts and technical course of study.

Each summer the **University of Wisconsin** sends a group of geology students to a new research area. This year 26 men, supervised by L. R. Laudon, will spend eight weeks in the Pentagon Mountain region (Mont.) and in the southern Canadian Rockies, collecting geological data.

Woods Hole Oceanographic Institution has sent a party of ten scientists, under the direction of Frederick C. Fuglister and Valentine Worthington, on a five months' cruise to survey the Gulf Stream between North America, Iceland, and England. Aboard the research vessel *Albatross III* are the usual drift bottles, a bathythermograph, a geomagnetic electrokinetograph (GEK, a device based on the law of magnetic induction), and a new underwater camera, which uses a timing mechanism that can be set to take pictures from once a second to once every five minutes.

Grants and Fellowships

The **American Philosophical Society,** through its Penrose Fund, has made a grant to Otto Struve for research in astrophysics. Other grants went to Ralph W. Chaney, Robert J. Drake, Ashley B. Gurney, Frank R. Shaw, Alice S. Baker, Howard E. Evans, Arthur

C. Cole, Jr., Myron Gordon, L. C. Dunn, Cornelis A. G. Wiersma, and Clement L. Markert, in the biological sciences; in sociology, to MacEdward Leach and Genevieve M. d'Haucourt; in archaeology, to Muriel N. Porter and Ephraim A. Speiser. Johnson Fund grants were made to Horace C. Richards (geology) and James A. G. Rehn (zoology). Nicholas A. Michels received a grant from the Daland Fund for research in anatomy.

Bristol Laboratories, of Syracuse, N. Y., has made a grant to Northwestern University Medical School for a fellowship in the Department of Biochemistry. The Fellow will work under the direction of E. A. Zeller. The University of Illinois College of Medicine has received support from Bristol for a research fellowship under the supervision of S. B. Binkley.

The **Building Service Employees International Union** has awarded $4,800 Columbia University scholarships to four children of its members. By 1954 the union expects to be maintaining 16 scholars at Columbia and Barnard. Four sons of New York City electrical workers will also enter Columbia next September as the winners of six-year scholarships awarded by the **Joint Industry Board,** contractors of the electrical industry, and **Local 3, International Brotherhood of Electrical Workers,** A. F. of L. When the plan is in full operation, 96 scholars will be studying at Columbia.

The **Eli Lilly Research Laboratories** have given $6,000 to Chicago Medical School for work in experimental diabetes under the direction of Piero P. Foa. The **Chicago Heart Association** has made grants to the school for studies in cardiology under the direction of Aldo A. Luisada, and in microbiology under George J. Scheff.

A **March of Dimes** grant of $19,400 will enable New York University, under the direction of Harold K. Work, to build a new, more efficient type of mechanical respirator. The new machine will provide respiration by negative, positive, and equalizing pressures, and will include a "mechanical cough" device now undergoing clinical trials at the university.

The **National Vitamin Foundation** has announced new grants-in-aid, ranging up to $9,600, to M. K. Horwitt, H. D. Wallace and T. J. Cunha, Nevis S. Scrimshaw, Robert W. Heinle and David R. Weir, Thomas P. Singer, E. W. McHenry, C. Lockard Conley, Albert E. Sobel, and Roberto Funaro. Grants are made semiannually, on January 1 and July 1.

New England Textile Foundation has awarded 41 undergraduate scholarships for 1951–52 at the four New England textile colleges: Bradford Durfee, Lowell, and New Bedford Textile Institutes, and the Textile School–Rhode Island School of Design.

The University of Wisconsin is accepting applications for its **Graduate Fellowship in Science Writing.** The fellowship, established in 1948, is for 10 months and carries a stipend of $1,100. The recipient should have a wide background in both physical and social sciences, some journalism training or experience, and should intend to take up science writing as a career. Applications, returnable by *August 15,* should be addressed to UW News Service, Observatory Hill Office Bldg., Madison 6. First holder of the fellowship, Kenneth Johnson, is now chief science writer, Central States Agricultural Experimental Station, Columbus, Ohio.

Meetings and Elections

The third meeting of the **Indo-Pacific Fisheries Council,** a division of FAO, was held in Madras, February 1–3. J. D. F. Hardenburg, of Indonesia, presided and K. M. Munshi, India's Minister for Food and Agriculture, inaugurated the conference. It was attended by delegates from the United Kingdom, Australia, Cambodia, France, Indonesia, the Netherlands, Pakistan, the Philippines, Thailand, Viet Nam, and India. Observers from Unesco and SCAP were also present. Dr. Hardenburg stressed the importance of international cooperation to solve the urgent problems facing the fishing industry in the region. He said that it was the aim of the council to alleviate the food shortage and he outlined methods of improving the catch, simultaneously warning against the danger of overfishing. He added that new branches of the industry must be created, markets built, and research and statistical information gathered. In view of the importance of inland fisheries in solving the food problem facing India, Mr. Munshi mentioned that the government had sanctioned 23 "Grow More Fish" schemes and had also provided technical assistance to the states. The development and extension of technical instruction for the fisheries people were discussed, and the feature of the meeting was a symposium on transplantation of fish, wherein great emphasis was laid on pond fishing and utilization of noncultivable lands for fish-rearing. The government of the Philippines has invited the council to hold its next meeting in Manila.

The **Royal Society of Canada** has elected H. E. Angus president and G. B. Reed vice president. C. Leonard Huskins, of the University of Wisconsin, was elected president of Section 5 (biological and medical sciences), and H. Carmichael, J. W. Hopkins, and W. G. Schneider were elected Fellows. W. G. Penfield, of the Faculty of Medicine, McGill University, was awarded the Society's Flavelle Medal.

At the annual meeting of **The Society for Investigative Dermatology,** the following officers were elected: president, Samuel M. Peck; vice president, John R. Haserick; director (five years), Marion B. Sulzberger.

The **Society of the Plastics Industry** has elected Gordon Brown president; Horace Gooch, Jr., chairman of the board; Dale Amos, vice president; and J. E. Gould, secretary-treasurer. The annual John Wesley Hyatt award was presented to James Bailey, vice president and director of research of the Plax Corporation, of Hartford, Conn.

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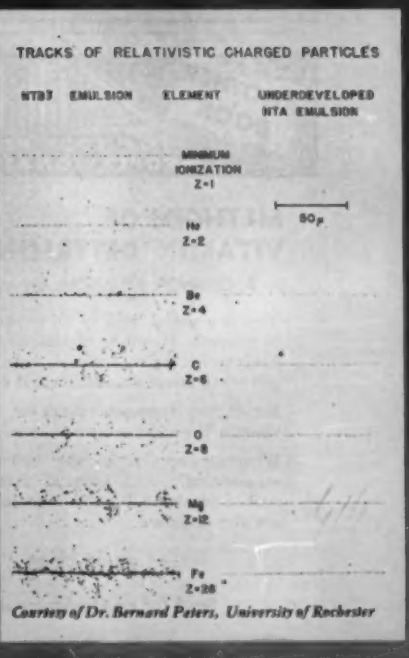

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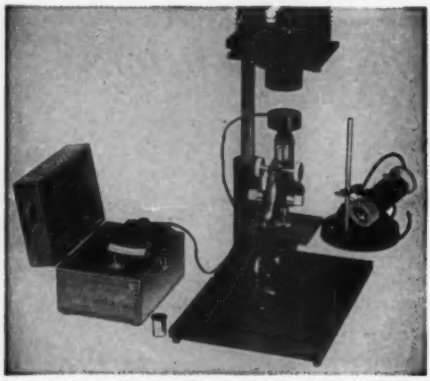

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July 23–26. Conference on Auroral Physics. University of Western Ontario, London, Can.
Aug. 6–12. British Association for the Advancement of Science. Edinburgh.
Aug. 7–9. American Meteorological Society. State College, Pa.
Aug. 13–Sept. 7. Canadian Mathematical Congress (Summer Seminar). Dalhousie University, Halifax.
Aug. 14–16. Conference on Prestressed Concrete. MIT, Cambridge, Mass.
Aug. 20–23. American Institute of Electrical Engineers (Pacific). Multnomah Hotel, Portland, Ore.
Aug. 20–23. National Council of Teachers of Mathematics (Annual). St. Olaf College, Northfield, Minn.
Aug. 22–25. Plant Science Seminar. University of Buffalo School of Pharmacy, Buffalo, N. Y.
Aug. 23–30. Institute for Teachers of Mathematics. Connecticut College, New London.
Aug. 26–31. American Pharmaceutical Association. Statler Hotel, Buffalo.
Aug. 27–29. American Association of Jesuit Scientists, Eastern States Division (Annual). Boston College, Chestnut Hill, Mass.
Aug. 27–30. Illuminating Engineering Society. Washington, D. C.
Aug. 27–30. Social Work Institute, including discussions on the Problem of Old Age. Valparaiso University, Valparaiso, Ind.
Aug. 27–31. American Society of Agronomy. Pennsylvania State College, State College.
Aug. 27–31. Soil Science Society of America. Pennsylvania State College, State College.
Aug. 31–Sept. 5. American Psychological Association. Hotel Sherman, Chicago.
Sept. 1–3. Astronomical League. University of North Carolina, Chapel Hill.
Sept. 3–4. Mathematical Association of America (Summer). University of Minnesota, Minneapolis.
Sept. 3–7. American Chemical Society (Annual). New York.
Sept. 4–6. Pacific Coast Gas Association (Annual). Fairmont Hotel, San Francisco.
Sept. 4–7. American Mathematical Society (Summer). University of Minnesota, Minneapolis.
Sept. 5. Calorimetry Conference. Pupin Physics Laboratory, Columbia University, New York.
Sept. 5–7. Mycological Society of America. University of Minnesota, Minneapolis.
Sept. 8–9. International Union of Pure and Applied Chemistry. New York.
Sept. 8–12. Ecological Society of America. University of Minnesota, Minneapolis.
Sept. 8–15. American Occupational Therapy Association (Annual). New Castle, N. H.
Sept. 9–14. International Gerontological Congress. Hotel Jefferson, St. Louis.
Sept. 9–14. International Society for the Welfare of Cripples (World Congress). Stockholm.
Sept. 10–12. American Institute of Biological Sciences (Annual). University of Minnesota, Minneapolis.
Sept. 10–13. International Congress of Pure and Applied Chemistry. New York.
Sept. 10–13. Congress of the International Union of Leather Chemists Societies. London.

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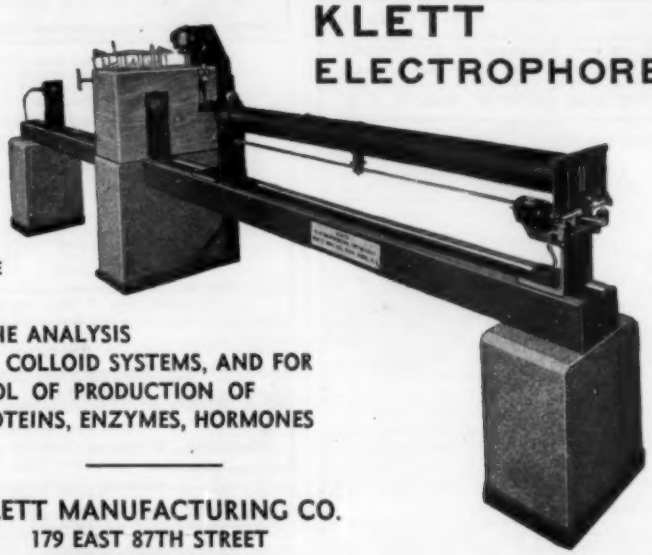

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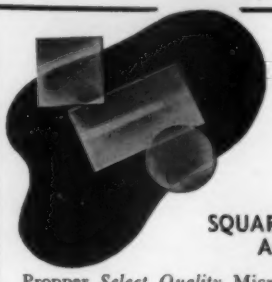

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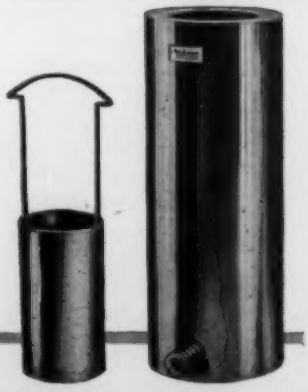

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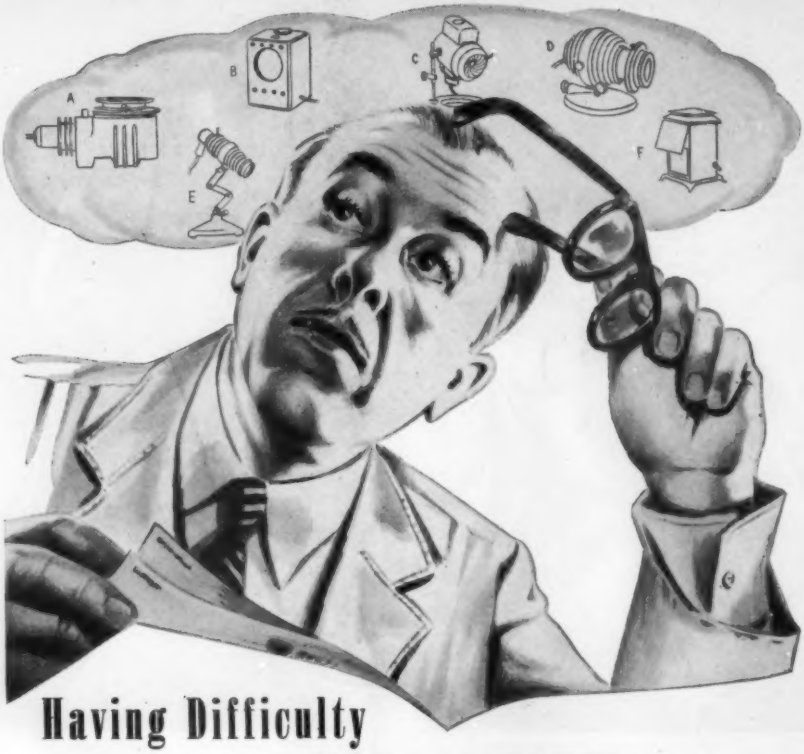

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